Spector, Lorrain

From: Spector, Lorraine
Sent: Tuesday, May 14, 2002 2:49 PM
To: Hutzell, Paula
Subj ct: RUSH SEARCH request for Serial No. 09/575199

Paula,
Would you please authorize the following RUSH search?
Reason: Amended, sequence only recently entered

STIC,
Serial Number:09/575199
Please search SEQ ID NO:2, residues 1-116.
-pending
-issued
-commercial

Thanks.

Lorraine Spector
703-308-1793
U.S. Patent and Trademark Office
Art Unit 1646
lorraine.spector@uspto.gov
CM1-10B11

```
    14may02 13:53:05 User217743 Session D554.1
       $0.00   0.177 DialUnits FileHomeBase
   $0.00  Estimated cost FileHomeBase
   $0.00  Estimated cost this search
   $0.00  Estimated total session cost   0.177 DialUnits
File 410:Chronolog(R) 1981-2002/May
    (c) 2002 The Dialog Corporation

    Set  Items  Description
    ---  -----  -----------
? set hi *;set hi *
HILIGHT set on as '*'*
*HILIGHT set on as '*'
? b 411
    14may02 13:53:08 User217743 Session D554.2
       $0.00   0.072 DialUnits File410
   $0.00  Estimated cost File410
   $0.01  TELNET
   $0.01  Estimated cost this search
   $0.01  Estimated total session cost   0.249 DialUnits
File 411:DIALINDEX(R)

DIALINDEX(R)
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*** DIALINDEX search results display in an abbreviated
*** *** format unless you enter the SET DETAIL ON
command. *** ? set files biochem
>>>       162 is unauthorized
>>>1 of the specified files is not available
  You have 25 files in your file list.
  (To see banners, use SHOW FILES command)
? s vegf and (disulfide or disulphide)

Your SELECT statement is:
  s vegf and (disulfide or disulphide)

      Items   File
      -----   ----
        23    5: Biosis Previews(R)_1969-2002/May W1
       26    34: SciSearch(R) Cited Ref
Sci_1990-2002/May W2              3    50: CAB
Abstracts_1972-2002/Apr
         15    71: ELSEVIER BIOBASE_1994-2002/May
W2
         21    73: EMBASE_1974-2002/May W1
         10    76: Life Sciences
Collection_1982-2002/May          1    94:
JICST-EPlus_1985-2002/Mar W4
          7    98: General Sci
Abs/Full-Text_1984-2002/Apr             4   144:
Pascal_1973-2002/May W2
         24   155: MEDLINE(R)_1966-2002/May W1
          1   156: ToxFile_1966-2002/Feb W4
          1   370: Science_1996-1999/Jul W3
         10   399: CA SEARCH(R)_1967-2002/UD=13620

  13 files have one or more items; file list includes 25
files.
? rf
Your last SELECT statement was:
  S VEGF AND (DISULFIDE OR DISULPHIDE)

Ref      Items   File
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N1          26    34: SciSearch(R) Cited Ref
Sci_1990-2002/May W2 N2           24   155:
MEDLINE(R)_1966-2002/May W1
N3          23     5: Biosis Previews(R)_1969-2002/May
W1 N4          21    73: EMBASE_1974-2002/May W1
N5          15    71: ELSEVIER BIOBASE_1994-2002/May
W2
N6          10    76: Life Sciences
Collection_1982-2002/May N7           10   399: CA
SEARCH(R)_1967-2002/UD=13620
N8           7    98: General Sci
Abs/Full-Text_1984-2002/Apr N9            4   144:
Pascal_1973-2002/May W2
N10          3    50: CAB Abstracts_1972-2002/Apr
  13 files have one or more items; file list includes 25
files.
      - Enter P or PAGE for more -
? s n2, n1

Your SELECT statement is:
  s n2, n1

      Items   File
      -----   ----

  No files have one or more items; file list includes 25
files.
? b n2,n1
    14may02 13:53:52 User217743 Session D554.3
       $0.89   0.508 DialUnits File411
   $0.89  Estimated cost File411
   $0.21  TELNET
   $1.10  Estimated cost this search
   $1.11  Estimated total session cost   0.757 DialUnits
SYSTEM:OS - DIALOG OneSearch
 File 155:MEDLINE(R) 1966-2002/May W1
*File 155: This file has been reloaded. Accession numbers
have changed.  File  34:SciSearch(R) Cited Ref Sci
1990-2002/May W2
      (c) 2002 Inst for Sci Info

    Set  Items  Description
    ---  -----  -----------
? s vegf and (disulfide or disulphide)
       11507  VEGF
       43199  DISULFIDE
```

6349 DISULPHIDE
S1 50 VEGF AND (DISULFIDE OR DISULPHIDE)
? red
>>>Unrecognizable Command
? rd
...examined 50 records (50)
...completed examining records
S2 27 RD (unique items)
? t s2/3,ab/all

2/3,AB/1 (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

13010801 21856773 PMID: 11866530
Solution structure of a phage-derived peptide antagonist in complex with vascular endothelial growth factor.
Pan Borlan; Li Bing; Russell Stephen J; Tom Jeffrey Y K; Cochran Andrea G ; Fairbrother Wayne J
Department of Protein Engineering, Genentech Inc., South San Francisco, CA 94080, USA.
Journal of molecular biology (England) Feb 22 2002, 316 (3) p769-87, ISSN 0022-2836 Journal Code: 2985088R
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Vascular endothelial growth factor (*VEGF*) is a potent endothelial cell-specific mediator of angiogenesis and vasculogenesis. *VEGF* is involved pathologically in cancer, proliferative retinopathy and rheumatoid arthritis, and as such represents an important therapeutic target. Three classes of *disulfide*-constrained peptides that antagonize binding of the *VEGF* dimer to its receptors, KDR and Flt-1, were identified previously using phage display methods. NMR studies of a representative peptide from the most potent class of these peptide antagonists, v107 (GGNECDAIRMWEWECFERL), were undertaken to characterize its interactions with *VEGF* . v107 has no defined structure free in solution, but binding to *VEGF* induces folding of the peptide. The solution structure of the *VEGF* receptor-binding domain-v107 complex was determined using 3940 (1970 per *VEGF* monomer) internuclear distance and 476 (238 per *VEGF* monomer) dihedral angle restraints derived from NMR data obtained using samples containing either (13)C/(15)N-labeled protein plus excess unlabeled peptide or (13)C/(15)N-labeled peptide plus excess unlabeled protein. Residual dipolar coupling restraints supplemented the structure determination of the complex and were found to increase significantly both the global precision of *VEGF* in the complex and the agreement with available crystal structures of *VEGF* . The calculated ensemble of structures is of high precision and is in excellent agreement with the experimental restraints. v107 has a turn-helix conformation with hydrophobic residues partitioned to one face of the peptide and polar or charged residues at the other face. Contacts between two v107 peptides and the *VEGF* dimer are mediated by primarily hydrophobic side-chain interactions. The v107-binding site on *VEGF* overlaps partially with the binding site of KDR and is similar to that for domain 2 of Flt-1. The structure of the *VEGF*-v107 complex provides new insight into how binding to *VEGF* can be achieved that may be useful for the design of small molecule antagonists. Copyright 2002 Elsevier Science Ltd.

2/3,AB/2 (Item 2 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

11005963 20555762 PMID: 11106176
Purification and refolding of vascular endothelial growth factor-B. Scrofani S D; Fabri L J; Xu P; Maccarone P; Nash A D
AMRAD Operations Pty Ltd, Richmond, Victoria, Australia. Protein science : a publication of the Protein Society (UNITED STATES) Oct 2000, 9 (10) p2018-25, ISSN 0961-8368 Journal Code: 9211750 Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Vascular endothelial growth factor (*VEGF*)-A interacts with the receptor tyrosine kinases *VEGF*-R1 and R2, and the importance of this interaction in endothelial cell (EC) function and blood vessel development has been well documented. Other ligands that interact differentially with these receptors and that are structurally related to *VEGF*-A include *VEGF*-B, *VEGF*-C, *VEGF* -D, and placenta growth factor (PLGF). Compared with *VEGF*-A, relatively little is known about the biological role of the *VEGF*-R1 specific ligand, *VEGF*-B. Two splice variant isoforms that differ at the COOH-terminus and which retain unique solubility characteristics are widely expressed throughout embryonic and postnatal development. Recent analysis of mice with a targeted deletion of the *VEGF* -B gene has revealed a defect in heart development and function consistent with an important role in vascularization of the myocardium (Bellomo D et al., 2000, Circ Res 86:E29-E35). To facilitate further characterization of *VEGF*-B, we have developed a protocol for expression and purification of refolded recombinant protein from Escherichia coli inclusion bodies (IBs). The approach developed resolves a number of significant issues associated with *VEGF*-B, including the ability to heterodimerize with endogenous *VEGF*-A when co-expressed in mammalian cells, a

complex secondary structure incorporating inter- and intrachain *disulfide* bonds and hydrophobic characteristics that preclude the use of standard chromatographic resins. The resulting purified *disulfide*-linked homodimer was demonstrated to bind to *VEGF*-R1 and to compete with *VEGF*-A for binding to this receptor.

2/3,AB/3 (Item 3 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10451552 99444104 PMID: 10512636
Vascular endothelial growth factor *VEGF* -like heparin-binding protein from the venom of Vipera aspis aspis (Aspic viper). Komori Y; Nikai T; Taniguchi K; Masuda K; Sugihara H
Department of Microbiology, Faculty of Pharmacy, Meijo University, 150 Yagotoyama, Tenpaku-ku, Nagoya, 468-8503, Japan.
Biochemistry (UNITED STATES) Sep 7 1999, 38 (36) p11796-803, ISSN 0006-2960 Journal Code: 0370623
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The heparin-binding dimeric hypotensive factor (HF) was purified from Vipera aspis aspis (Aspic viper) venom [Komori, Y. and Sugihara, H. (1990) Toxicon 28, 359-369]. In this study, the amino acid sequence, and structure and function of HF, were elucidated. By electrospray ionization mass spectrometry (ESI-MS), the molecular weight of HF was determined to be 25 072.1. The complete amino acid sequence of HF was determined by Edman sequencing of the S-pyridylethylated HF and its peptides derived from enzymatic digestion. The theoretical molecular mass calculated from the primary structure agrees well with the molecular weight determined by ESI-MS. HF consists of two homogeneous monomers bound covalently. The monomer with an N-terminal blocked by pyroglutamic acid contains 110 amino acid residues, including eight cysteine residues, two of which are considered to be involved in intermolecular *disulfide* bonds. Sequential homology search revealed that the primary structure of HF is similar to that of vascular endothelial growth factor (*VEGF*) and platelet-derived growth factor (PDGF) with a sequential homology of 45 and 22%, respectively. When injected intradermally into a rat, an increase in capillary permeability was observed with HF or *VEGF*. On the other hand, only HF exerted a strong hypotensive effect after intravenous injection of samples into a rat. Purified HF has a mitogenic effect on endothelial cells. Through the use of bovine aortic endothelial cells (BAEC), the half-maximal mitogenic concentration of HF was determined to be 5-5.5 nM (125-138 ng/mL). Similarly, *VEGF* had a mitogenic concentration at 0.5-1 nM. When incubated with HF and cycloheximide or HF and heparin, the cell growth was inhibited, suggesting that the mechanism of action of HF is similar to that of *VEGF*.

2/3,AB/4 (Item 4 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10204368 99179016 PMID: 10077638
Vascular endothelial growth factor (*VEGF*)-like protein from orf virus NZ2 binds to VEGFR2 and neuropilin-1.
Wise L M; Veikkola T; Mercer A A; Savory L J; Fleming S B; Caesar C; Vitali A; Makinen T; Alitalo K; Stacker S A
Virus Research Unit, Department of Microbiology, University of Otago, P.O. Box 56, Dunedin, New Zealand.
Proceedings of the National Academy of Sciences of the United States of America (UNITED STATES) Mar 16 1999, 96 (6) p3071-6, ISSN 0027-8424 Journal Code: 7505876
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Orf virus, a member of the poxvirus family, produces a pustular dermatitis in sheep, goats, and humans. The lesions induced after infection with orf virus show extensive proliferation of vascular endothelial cells, dilation of blood vessels and dermal swelling. An explanation for the nature of these lesions may lie in the discovery that orf virus encodes an apparent homolog of the mammalian vascular endothelial growth factor (*VEGF*) family of molecules. These molecules mediate endothelial cell proliferation, vascular permeability, angiogenesis, and lymphangiogenesis via the endothelial cell receptors VEGFR-1 (Flt1), VEGFR-2 (KDR/Flk1), and VEGFR-3 (Flt4). The *VEGF*-like protein of orf virus strain NZ2 (ORFV2- *VEGF*) is most closely related in primary structure to *VEGF*. In this study we examined the biological activities and receptor specificity of the ORFV2-*VEGF* protein. ORFV2-*VEGF* was found to be a *disulfide* -linked homodimer with a subunit of approximately 25 kDa. ORFV2-*VEGF* showed mitogenic activity on bovine aortic and human microvascular endothelial cells and induced vascular permeability. ORFV2- *VEGF* was found to bind and induce autophosphorylation of VEGFR-2 and was unable to bind or activate VEGFR-1 and VEGFR-3, but bound the newly identified VEGF165 receptor neuropilin-1. These results indicate that, from a functional viewpoint, ORFV2-*VEGF* is indeed a member of the *VEGF* family of molecules, but is unique, however, in that it utilizes only VEGFR-2 and neuropilin-1.

2/3,AB/5 (Item 5 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10125708 99119203 PMID: 9922141
Novel peptides selected to bind vascular endothelial growth factor target the receptor-binding site.
Fairbrother W J; Christinger H W; Cochran A G; Fuh G; Keenan C J; Quan C; Shriver S K; Tom J Y; Wells J A; Cunningham B C
Department of Protein Engineering, Genentech, Inc., South San Francisco, California 94080, USA.
Biochemistry (UNITED STATES) Dec 22 1998, 37 (51) p17754-64, ISSN 0006-2960 Journal Code: 0370623
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Peptides that inhibit binding of vascular endothelial growth factor (*VEGF*) to its receptors, KDR and Flt-1, have been produced using phage display. Libraries of short *disulfide*-constrained peptides yielded three distinct classes of peptides that bind to the receptor-binding domain of *VEGF* with micromolar affinities. The highest affinity peptide was also shown to antagonize *VEGF*-induced proliferation of primary human umbilical vascular endothelial cells. The peptides bind to a region of *VEGF* known to contain the contact surface for Flt-1 and the functional determinants for KDR binding. This suggests that the receptor-binding region of *VEGF* is a binding "hot spot" that is readily targeted by selected peptides and supports earlier assertions that phage-derived peptides frequently target protein-protein interaction sites. Such peptides may lead to the development of pharmacologically useful *VEGF* antagonists.

2/3,AB/6 (Item 6 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10018540 99009074 PMID: 9792673
SPARC (BM-40, osteonectin) inhibits the mitogenic effect of vascular endothelial growth factor on microvascular endothelial cells. Kupprion C; Motamed K; Sage E H
Department of Biological Structure, University of Washington School of Medicine, Seattle, Washington 98195-7420, USA.
Journal of biological chemistry (UNITED STATES) Nov 6 1998, 273 (45) p29635-40, ISSN 0021-9258 Journal Code: 2985121R
Contract/Grant No.: GM 40711; GM; NIGMS; HL 18645; HL; NHLBI Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
SPARC (secreted protein, acidic and rich in cysteine) is a matricellular protein that modulates cell adhesion and proliferation and is thought to function in tissue remodeling and angiogenesis. In this study, we demonstrate that SPARC inhibits DNA synthesis by >90% in human microvascular endothelial cells (HMEC) stimulated by the endothelial cell mitogen vascular endothelial growth factor (*VEGF*). Peptides derived from SPARC domain IV, which contains a *disulfide*-bonded EF-hand sequence and binds to endothelial cells, mimicked the effect of native SPARC. The inhibition was also observed with a peptide from the follistatin-like domain II, whereas peptides from SPARC domains I and III had no effect on *VEGF*-stimulated DNA synthesis. The inhibition of HMEC proliferation was mediated in part by the binding of *VEGF* to SPARC. The binding of 125I-*VEGF* to HMEC was reduced by SPARC and SPARC peptides from domain IV in a concentration-dependent manner. In a radioimmune precipitation assay, peptides from SPARC domains II and IV each competed with native SPARC for its binding to *VEGF*. It has been reported that *VEGF* stimulates the tyrosine phosphorylation and activation of mitogen-activated protein kinases Erk1 and Erk2. We now show that SPARC reduces this phosphorylation in *VEGF*-stimulated HMEC to levels of unstimulated controls. SPARC thus modulates the mitogenic activity of *VEGF* through a direct binding interaction and reduces the association of *VEGF* with its cell-surface receptors. Moreover, an additional diminution of *VEGF* activity by SPARC is accomplished through a reduction in the tyrosine phosphorylation of mitogen-activated protein kinases.

2/3,AB/7 (Item 7 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09872954 98298440 PMID: 9634701
Solution structure of the heparin-binding domain of vascular endothelial growth factor.
Fairbrother W J; Champe M A; Christinger H W; Keyt B A; Starovasnik M A Department of Protein Engineering, Genentech, Inc. fairbro@gene.com Structure (London, England) (ENGLAND) May 15 1998, 6 (5) p637-48, ISSN 0969-2126 Journal Code: 9418985
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
BACKGROUND: Vascular endothelial growth factor (*VEGF*) is an endothelial cell-specific mitogen and is a potent angiogenic and vascular permeabilizing factor.

VEGF is also an important mediator of pathological angiogenesis associated with cancer, rheumatoid arthritis and proliferative retinopathy. The binding of *VEGF* to its two known receptors, KDR and Flt-1, is modulated by cell-surface-associated heparin-like glycosaminoglycans and exogenous heparin or heparan sulfate. Heparin binding to VEGF165, the most abundantly expressed isoform of *VEGF*, has been localized to the carboxy-terminal 55 residues; plasmin cleavage of VEGF165 yields a homodimeric 110-residue amino-terminal receptor-binding domain (VEGF110) and two 55-residue carboxy-terminal heparin-binding fragments. The endothelial cell mitogenic potency of VEGF110 is decreased significantly relative to VEGF165, indicating that the heparin-binding domains are critical for stimulating endothelial cell proliferation. RESULTS: The solution structure of the 55-residue heparin-binding domain of VEGF165 has been solved using data from two-dimensional homonuclear and three-dimensional heteronuclear NMR spectroscopy. The structure has two subdomains, each containing two *disulfide* bridges and a short two-stranded antiparallel beta sheet; the carboxy-terminal subdomain also contains a short alpha helix. Hydrophobic interactions are limited to sidechains packing against the *disulfide* bridges. CONCLUSIONS: The heparin-binding domain of *VEGF* has no significant sequence or structural similarity to any known proteins and thus represents a novel heparin-binding domain. Most of the positively charged amino acid sidechains are localized on one side of the carboxy-terminal subdomain or on an adjacent disordered loop in the amino-terminal subdomain. The observed distribution of surface charges suggests that these residues constitute a heparin interaction site.

2/3,AB/8 (Item 8 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09744150 98175915 PMID: 9506953
A recombinant mutant vascular endothelial growth factor-C that has lost vascular endothelial growth factor receptor-2 binding, activation, and vascular permeability activities.
Joukov V; Kumar V; Sorsa T; Arighi E; Weich H; Saksela O; Alitalo K Molecular/Cancer Biology Laboratory, Haartman Institute, PL 21 Haartmaninkatu 3, University of Helsinki, 00014 Helsinki, Finland. Journal of biological chemistry (UNITED STATES) Mar 20 1998, 273 (12) p6599-602, ISSN 0021-9258 Journal Code: 2985121R
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The vascular endothelial growth factor (*VEGF*) and the *VEGF*-C promote growth of blood vessels and lymphatic vessels, respectively. *VEGF* activates the endothelial *VEGF* receptors (VEGFR) 1 and 2, and *VEGF*-C activates VEGFR-3 and VEGFR-2. Both *VEGF* and *VEGF* -C are also potent vascular permeability factors. Here we have analyzed the receptor binding and activating properties of several cysteine mutants of *VEGF*-C including those (Cys156 and Cys165), which in other platelet-derived growth factor/*VEGF* family members mediate interchain *disulfide* bonding. Surprisingly, we found that the recombinant mature *VEGF* -C in which Cys156 was replaced by a Ser residue is a selective agonist of VEGFR-3. This mutant, designated DeltaNDeltaC156S, binds and activates VEGFR-3 but neither binds VEGFR-2 nor activates its autophosphorylation or downstream signaling to the ERK/MAPK pathway. Unlike *VEGF* -C, DeltaNDeltaC156S neither induces vascular permeability in vivo nor stimulates migration of bovine capillary endothelial cells in culture. These data point out the critical role of VEGFR-2-mediated signal transduction for the vascular permeability activity of *VEGF*-C and strongly suggest that the redundant biological effects of *VEGF* and *VEGF* -C depend on binding and activation of VEGFR-2. The DeltaNDeltaC156S mutant may provide a valuable tool for the analysis of *VEGF* -C effects mediated selectively via VEGFR-3. The ability of DeltaNDeltaC156S to form homodimers also emphasizes differences in the structural requirements for *VEGF* and *VEGF*-C dimerization.

2/3,AB/9 (Item 9 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09635811 98047223 PMID: 9388030
Novel antibodies directed against the extracellular domain of the human *VEGF*-receptor type II.
Menrad A; Thierauch K H; Martiny-Baron G; Siemeister G; Schirner M; Schneider M R
Research Laboratories of Schering AG, Berlin, Germany.
Hybridoma (UNITED STATES) Oct 1997, 16 (5) p465-71, ISSN 0272-457X Journal Code: 8202424
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Vascular endothelial cell growth factor (*VEGF*) plays a pivotal role in the regulation of angiogenesis by binding to its cognate receptor molecule type II (VEGFr-II, KDR). VEGFr-II is an endothelial cell-specific transmembrane tyrosine kinase important for vascular endothelial cell development and differentiation during embryogenesis, angiogenic processes under physiological conditions, and various diseases. An increasing number of reports indicate that *VEGF*/VEGFr-II also play a fundamental role for tumor angiogenesis. We

present the generation and in vitro characterization of the monoclonal antibodies 2-7-9 and 2-10-1. Both antibodies are highly specific for VEGFr-II as demonstrated by Western blotting and immunoprecipitation. MAbs 2-10-1 and 2-7-9 bind to a *disulphide* bridge-stabilized epitope within domains 6 and 7 of the human VEGFr-II with an affinity of 8 and 80 nM, respectively. Furthermore, the antibodies are suitable for immunohistochemistry and ELISA techniques. Because both antibodies recognize their epitope on living cells, they also have the potential for drug targeting and diagnostic purposes.

2/3,AB/10 (Item 10 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09477342 97386538 PMID: 9244387
Disulfide structure of the heparin binding domain in vascular endothelial growth factor: characterization of posttranslational modifications in *VEGF*.
Keck R G; Berleau L; Harris R; Keyt B A
Department of Analytical Chemistry, Genentech, Inc., South San Francisco, California 94080, USA.
Archives of biochemistry and biophysics (UNITED STATES) Aug 1 1997, 344 (1) p103-13, ISSN 0003-9861 Journal Code: 0372430 Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Preparations of recombinant human vascular endothelial growth factor (VEGF165) expressed in Chinese hamster ovary (CHO) cells and Escherichia coli were compared using a variety of analytical methods. Amino terminal sequence analyses of both the CHO- and E. coli-derived VEGF165 confirmed the predicted amino terminal sequence for VEGF165, although the CHO VEGF165 exhibited a heterogeneous amino terminus with sequences beginning at Ala-1 (76%), Pro-2 (4%), Ala-4 (13%), and Glu-5 (7%). Tryptic digests of reduced and carboxymethylated CHO- and E. coli-derived VEGF165 were examined by LC/MS analyses, indicating equivalent primary structure, except for the glycosylation at Asn-75 in the CHO-derived VEGF165. The N-linked carbohydrate in the CHO-derived VEGF165 was determined to be a complex fucosylated biantennary structure. The data obtained from LC/MS analysis and amino terminal sequence analysis of VEGF165 confirmed 98% of the primary structure. *Disulfide* linkages for the eight cysteine residues in the carboxyl terminal heparin binding domain were assigned by amino terminal sequencing of fragments produced by tryptic digests of each native molecule. The following disulfides have been identified for both CHO- and E. coli-derived VEGF165: Cys-117 and Cys-135, Cys-120 and Cys-137, Cys-139 and Cys-158, plus Cys-146 and Cys-160. Plasmin cleavage of VEGF165 yields an N-terminal homodimeric VEGF110 and a 55-amino-acid carboxyl terminal domain. VEGF110 was resistant to further proteolytic or chemical digestion such that the *disulfide* linkages were not elucidated. The 55-amino-acid carboxyl terminal region of VEGF165 appears to be a unique heparin binding domain with no known protein homology.

2/3,AB/11 (Item 11 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09453226 97337423 PMID: 9194163
Computer modelling of the receptor-binding domains of *VEGF* and PIGF.
Walsh T P; Grant G H
Department of Biochemistry, University College, Belfield, Dublin, Republic of Ireland.
Protein engineering (ENGLAND) Apr 1997, 10 (4) p389-98, ISSN 0269-2139 Journal Code: 8801484
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Models of the platelet-derived growth factor (PDGF)-like domains of vascular endothelial growth factor (*VEGF*) and placenta growth factor (PIGF) were built based on their homology to PDGF. These domains contain most of the determinants for receptor binding. The sequences of these proteins exhibit limited but significant homology to that of platelet-derived growth factor (PDGF), a member of the cystine knot growth factor family. The eight cysteine residues that are involved in intra- and interchain *disulphide* bonds are conserved. Two high affinity receptors for *VEGF* have been identified, only one of which binds PIGF. The models presented here are consistent with results that show that *VEGF* receptor binding is mediated by charged residues in the loops. A comparison of the models suggests that the difference in receptor-binding specificity between *VEGF* and PIGF may be due to differences in the distribution of positively charged residues and the exposure of hydrophobic residues in the loops.

2/3,AB/12 (Item 12 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09434269 97337837 PMID: 9194531
A novel regulatory function of proteolytically cleaved *VEGF*-2 for vascular endothelial and smooth muscle cells.
Hu J S; Hastings G A; Cherry S; Gentz R; Ruben S; Coleman T A Department of Protein Therapeutics,

Human Genome Sciences, Inc., Rockville, Maryland 20850, USA.

FASEB journal : official publication of the Federation of American Societies for Experimental Biology (UNITED STATES) May 1997, 11 (6) p498-504, ISSN 0892-6638 Journal Code: 8804484

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

By high throughput sequencing, we have identified a cDNA encoding a polypeptide related to vascular endothelial growth factor (*VEGF*) and placenta growth factor (PlGF) in the *VEGF*/PDGF gene family. It is designated vascular endothelial growth factor 2 (*VEGF*-2). Similar to *VEGF*, expression of *VEGF*-2 mRNA is abundant in vascular smooth muscle cells and several highly vascularized tissues. *VEGF*-2 protein is expressed as a secreted 52 kDa precursor as well as the 30 kDa amino-terminal and 27 kDa carboxy-terminal cleavage products. The latter two cleavage products are linked via a *disulfide* bridge (or bridges) and can be copurified. Using copurified 30 and 27 kDa proteins, the effect of *VEGF* -2 on growth of several cell types, including vascular endothelial and smooth muscle cells, was determined. Our results demonstrate that *VEGF* -2 protein stimulates the growth of human vascular endothelial cells but inhibits growth of human aortic smooth muscle cells induced by platelet-derived growth factor. These studies establish *VEGF* -2 as a novel regulator for growth of vascular endothelial and smooth muscle cells.

2/3,AB/13 (Item 13 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09256294 97164697 PMID: 9012504

VEGF -C receptor binding and pattern of expression with VEGFR-3 suggests a role in lymphatic vascular development.

Kukk E; Lymboussaki A; Taira S; Kaipainen A; Jeltsch M; Joukov V; Alitalo K

Molecular/Cancer Biology Laboratory, Haartman Institute, University of Helsinki, Finland.

Development (Cambridge, England) (ENGLAND) Dec 1996, 122 (12) p3829-37, ISSN 0950-1991 Journal Code: 8701744

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

The vascular endothelial growth factor family has recently been expanded by the isolation of two new *VEGF*-related factors, *VEGF*-B and *VEGF* -C. The physiological functions of these factors are largely unknown. Here we report the cloning and characterization of mouse *VEGF*-C, which is produced as a *disulfide*-linked dimer of 415 amino acid residue polypeptides, sharing an 85% identity with the human *VEGF*-C amino acid sequence. The recombinant mouse *VEGF*-C protein was secreted from transfected cells as VEGFR-3 (Flt4) binding polypeptides of 30-32x10(3) Mr and 22-23x10(3) Mr which preferentially stimulated the autophosphorylation of VEGFR-3 in comparison with VEGFR-2 (KDR). In in situ hybridization, mouse *VEGF*-C mRNA expression was detected in mesenchymal cells of postimplantation mouse embryos, particularly in the regions where the lymphatic vessels undergo sprouting from embryonic veins, such as the perimetanephric, axillary and jugular regions. In addition, the developing mesenterium, which is rich in lymphatic vessels, showed strong *VEGF*-C expression. *VEGF*-C was also highly expressed in adult mouse lung, heart and kidney, where VEGFR-3 was also prominent. The pattern of expression of *VEGF*-C in relation to its major receptor VEGFR-3 during the sprouting of the lymphatic endothelium in embryos suggests a paracrine mode of action and that one of the functions of *VEGF*-C may be in the regulation of angiogenesis of the lymphatic vasculature.

2/3,AB/14 (Item 14 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09074381 96439123 PMID: 8841458

Activation-induced expression of vascular permeability factor by human peripheral T cells: a non-radioisotopic semiquantitative reverse transcription-polymerase chain reaction assay.

Iijima K; Yoshikawa N; Nakamura H

Department of Pediatrics, Kobe University School of Medicine, Japan. Journal of immunological methods (NETHERLANDS) Sep 27 1996, 196 (2) p199-209, ISSN 0022-1759 Journal Code: 1305440

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Vascular permeability factor, also known as vascular endothelial growth factor (VPF/*VEGF*), is a *disulfide*-linked dimeric glycoprotein of about 40 kDa that enhances vascular permeability, induces chemotaxis and activation of monocytes/macrophages, and promotes growth of vascular endothelial cells. It has been reported that several tumor cell lines and normal cells produce VPF/*VEGF*. To examine the possibility of VPF/ *VEGF* mRNA expression in human peripheral T cells and its mechanism(s) of regulation, we developed a non-radioisotopic semiquantitative reverse

transcription-polymerase chain reaction (RT-PCR; VPF/*VEGF* , GAPDH co-amplification) assay which detects all species of VPF/*VEGF* mRNA alternative splicing products. T cells expressed negligible VPF/*VEGF* mRNA in the resting state. However, TPA markedly enhanced the expression of 121-, 165- and 189-amino-acid-containing isoforms of VPF/*VEGF* mRNA in T cells. Both CD4+ and CD8+ T cells expressed VPF/*VEGF* mRNA in response to TPA treatment. Moreover, T cell receptor stimulation with monoclonal anti-CD3 antibody with or without IL-1 beta enhanced VPF/ *VEGF* mRNA expression in T cells. These findings suggest that T cell activation induces VPF/*VEGF* expression in the cells, resulting in induction of its biological activities.

2/3,AB/15 (Item 15 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08963966 96325041 PMID: 8702615
Genomic organization of the mouse and human genes for vascular endothelial growth factor B (*VEGF*-B) and characterization of a second splice isoform.
Olofsson B; Pajusola K; von Euler G; Chilov D; Alitalo K; Eriksson U Ludwig Institute for Cancer Research, Stockholm Branch, Box 240, S-171 77 Stockholm, Sweden.
Journal of biological chemistry (UNITED STATES) Aug 9 1996, 271 (32) p19310-7, ISSN 0021-9258 Journal Code: 2985121R
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
A second isoform and the genomic structures of mouse and human vascular endothelial growth factor B are described. Both genes consist of seven coding exons and span about 4 kilobases of DNA. The two identified isoforms of vascular endothelial growth factor B are generated by alternative splicing where different splice acceptor sites in exon 6 introduce a frameshift and a partial use of different but overlapping reading frames. Consequently, the COOH-terminal domains in the two isoforms show no resemblance. Mouse and human cDNA clones for the novel isoform of vascular endothelial growth factor B encoded a secreted protein of 186 amino acid residues. Expression in transfected cells generated a protein of 25 kDa which upon secretion was modified by O-linked glycosylation and displayed a molecular mass of 32 kDa under reducing conditions. The protein was expressed as a *disulfide* -linked homodimer, and heterodimers were generated when coexpressed with vascular endothelial growth factor. The entirely different COOH-terminal domains in the two isoforms of vascular endothelial growth factor B imply that some functional properties of the two proteins are distinct.

2/3,AB/16 (Item 16 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08883603 96222489 PMID: 8670191
Expression of biologically active isoforms of the tumor angiogenesis factor *VEGF* in Escherichia coli.
Siemeister G; Schnurr B; Mohrs K; Schachtele C; Marme D; Martiny-Baron G Tumor Biology Center, Institute of Molecular Medicine, Freiburg Federal Republic of Germany.
Biochemical and biophysical research communications (UNITED STATES) May 15 1996, 222 (2) p249-55, ISSN 0006-291X Journal Code: 0372516 Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Vascular endothelial growth factor (*VEGF*) was identified as an endothelial cell specific mitogen that induces angiogenesis and vascular permeability in vivo. *VEGF* is a homodimeric protein which contains three intramolecular and two intermolecular *disulfide* bridges. Here, we report on an efficient procedure for recombinant production of *VEGF* isoforms VEGF121 and VEGF165 in Escherichia coli. The proteins were solubilized from inclusion bodies, refolded, and purified by chromatographic methods. The final protein products were almost completely in the dimeric conformation, bound to *VEGF* receptor FLT1 with a Kd of 30 pM, stimulated proliferation of human umbilical vein endothelial cells half-maximally at a concentration of 30 pM, and induced in vivo neovascularization and vascular permeability on the chicken chorioallantoic membrane.

2/3,AB/17 (Item 17 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08855385 96197355 PMID: 8637916
Vascular endothelial growth factor B, a novel growth factor for endothelial cells.
Olofsson B; Pajusola K; Kaipainen A; von Euler G; Joukov V; Saksela O; Orpana A; Pettersson R F; Alitalo K; Eriksson U
Ludwig Institute for Cancer Research, Stockholm, Sweden. Proceedings of the National Academy of Sciences of the United States of America (UNITED STATES) Mar 19 1996, 93 (6) p2576-81, ISSN 0027-8424 Journal Code: 7505876
Document type: Journal Article
Languages: ENGLISH

Main Citation Owner: NLM
Record type: Completed
We have isolated and characterized a novel growth factor for endothelial cells, vascular endothelial growth factor B (*VEGF*-B), with structural similarities to vascular endothelial growth factor (*VEGF*) and placenta growth factor. *VEGF*-B was particularly abundant in heart and skeletal muscle and was coexpressed with *VEGF* in these and other tissues. *VEGF*-B formed cell-surface-associated *disulfide*-linked homodimers and heterodimerized with *VEGF* when coexpressed. Conditioned medium from transfected 293EBNA cells expressing *VEGF*-B stimulated DNA synthesis in endothelial cells. Our results suggest that *VEGF* -B has a role in angiogenesis and endothelial cell growth, particularly in muscle.

2/3,AB/18 (Item 18 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08677981 96022601 PMID: 8527160
Expression of biologically active human vascular endothelial growth factor in yeast.
Mohanraj D; Olson T; Ramakrishnan S
Department of Pharmacology, University of Minnesota, Minneapolis 55455, USA.
Growth factors (Chur, Switzerland) (SWITZERLAND) 1995, 12 (1) p17-27 , ISSN 0897-7194 Journal Code: 9000468
Contract/Grant No.: CA-48608; CA; NCI
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Vascular endothelial growth factor (*VEGF*) is a glycoprotein consisting of two identical polypeptide chains linked by a *disulfide* bond. The unique biological activities of *VEGF* include its potent mitogenic and permeability inducing properties specific for the vascular endothelium. *VEGF* is implicated in tumor angiogenesis, wound healing, and the stimulation of collateral vessel formation at the site of arterial occlusion. Therefore, in order to produce large quantities of biologically active *VEGF*, a splice variant (VEGF165) was cloned and expressed in a yeast expression system. The coding region of VEGF165 was isolated from U937 cells by RT-PCR, sequenced and then cloned into the yeast expression vector pHILS1. VEGF165 was secreted into the medium as a dimer. Recombinant *VEGF* reacted to antibodies raised against the N-terminal and C-terminal synthetic polypeptides of human *VEGF*. As much as 35-40 mg/L of purified *VEGF* could be obtained from the yeast expression system. The recombinant protein was biologically active in inducing vascular endothelial cell proliferation in vitro and permeability changes in vivo.

2/3,AB/19 (Item 19 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08346657 95105171 PMID: 7806514
Covalent dimerization of vascular permeability factor/vascular endothelial growth factor is essential for its biological activity. Evidence from Cys to Ser mutations.
Potgens A J; Lubsen N H; van Altena M C; Vermeulen R; Bakker A; Schoenmakers J G; Ruiter D J; de Waal R M
Institute of Pathology, University Hospital Nijmegen, The Netherlands. Journal of biological chemistry (UNITED STATES) Dec 30 1994, 269 (52) p32879-85, ISSN 0021-9258 Journal Code: 2985121R
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Vascular permeability factor, or vascular endothelial growth factor (VPF/ *VEGF*) is an important factor in the regulation of vascular growth and vascular permeability. VPF is a secreted, dimeric protein and has 8 cysteine residues conserved with platelet-derived growth factor (PDGF). To study the role of some of these cysteine residues in maintaining the structure and function of VPF, we replaced the codons for the second, third, fourth, and fifth cysteine by serine codons, and expressed the mutant proteins in a mammalian expression system. Cysteine residues 2 and 4 in VPF were found to be directly involved in anti-parallel interchain *disulfide* bonds, as in PDGF. VPF mutants lacking one of these cysteins were severely impaired in their S-linked dimerization, while upon coexpression of both mutants the ability to form dimers was restored. The VPF mutants lacking cysteine residue 2 or 4 also competed poorly for receptor binding of labeled VPF and had low biological activity, but these defects were also complemented by coexpressing the two mutants, indicating that for efficient receptor binding and activation VPF needs to be a covalent dimer, unlike PDGF-BB. Furthermore, cysteine residue 5 was found to be essential for VPF dimerization and activity, while the mutant lacking cysteine residue 3 was only mildly affected in its ability to dimerize and had partial biological activity.

2/3,AB/20 (Item 20 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07969501 94108980 PMID: 8281615

Vascular permeability factor (VPF, *VEGF*) in tumor biology. Senger D R; Van de Water L; Brown L F; Nagy J A; Yeo K T; Yeo T K; Berse B; Jackman R W; Dvorak A M; Dvorak H F

Department of Pathology, Beth Israel Hospital, Boston, MA. Cancer and metastasis reviews (UNITED STATES) Sep 1993, 12 (3-4) p303-24, ISSN 0891-9992 Journal Code: 8605731

Contract/Grant No.: CA-40624; CA; NCI; CA-43967; CA; NCI; GM-36812; GM; NIGMS; +

Document type: Journal Article; Review; Review, Tutorial

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Vascular permeability factor (VPF), also known as vascular endothelial growth factor (*VEGF*), is a multifunctional cytokine expressed and secreted at high levels by many tumor cells of animal and human origin. As secreted by tumor cells, VPF/*VEGF* is a 34-42 kDa heparin-binding, dimeric, *disulfide* -bonded glycoprotein that acts directly on endothelial cells (EC) by way of specific receptors to activate phospholipase C and induce [Ca2+]i transients. Two high affinity VPF/ *VEGF* receptors, both tyrosine kinases, have thus far been described. VPF/*VEGF* is likely to have a number of important roles in tumor biology related, but not limited to, the process of tumor angiogenesis. As a potent permeability factor, VPF/*VEGF* promotes extravasation of plasma fibrinogen, leading to fibrin deposition which alters the tumor extracellular matrix. This matrix promotes the ingrowth of macrophages, fibroblasts, and endothelial cells. Moreover, VPF/*VEGF* is a selective endothelial cell (EC) growth factor in vitro, and it presumably stimulates EC proliferation in vivo. Furthermore, VPF/*VEGF* has been found in animal and human tumor effusions by immunoassay and by functional assays and very likely accounts for the induction of malignant ascites. In addition to its role in tumors, VPF/*VEGF* has recently been found to have a role in wound healing and its expression by activated macrophages suggests that it probably also participates in certain types of chronic inflammation. VPF/*VEGF* is expressed in normal development and in certain normal adult organs, notably kidney, heart, adrenal gland and lung. Its functions in normal adult tissues are under investigation.

2/3,AB/21 (Item 21 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07953497 94087986 PMID: 8264155

Human mesangial cells and peripheral blood mononuclear cells produce vascular permeability factor.

Iijima K; Yoshikawa N; Connolly D T; Nakamura H

Department of Pediatrics, Kobe University School of Medicine, Japan. Kidney international (UNITED STATES) Nov 1993, 44 (5) p959-66, ISSN 0085-2538 Journal Code: 0323470

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Vascular permeability factor, or vascular endothelial growth factor (VPF/ *VEGF*) is a *disulfide*-linked dimeric glycoprotein of about 40 kD that promotes fluid and protein leakage from blood vessels. Various human tumor cell lines and cells including fetal vascular smooth muscle cells produce VPF/*VEGF* . Since glomerular mesangial cells (MC) are closely related to vascular smooth muscle cells, we examined whether cultured human MC produce VPF/*VEGF* . Northern blotting analysis revealed that cultured human MC expressed a 3.7 kilobases (kb) VPF/*VEGF* mRNA. Human peripheral blood mononuclear cells (PBMC) also expressed VPF/*VEGF* transcripts of 8.6 and 3.8 kb. Although the sizes of the transcripts suggested the existence of unique molecular species of VPF/*VEGF* mRNA in PBMC, RT-PCR analysis revealed that PBMC as well as human MC expressed 121, 165, and 189 amino acid-containing isoforms of VPF/*VEGF*, implying that there are no unique alternative splicing products of VPF/ *VEGF* mRNA in PBMC. Fetal calf serum and 12-o-tetradecanoyl- phorbol-13-acetate (TPA) transiently enhanced VPF/*VEGF* mRNA expression in cultured human MC. Transforming growth factor-beta 1 enhanced VPF/*VEGF* mRNA expression in cultured human MC at least within 24 hours. Dexamethasone (DEX) inhibited the TPA-induced increase in VPF/ *VEGF* mRNA expression, whereas DEX did not change the basal level. The DEX depressed the TPA-induced increase in VPF/*VEGF* mRNA expression is therefore probably a result of transcriptional control. VPF/*VEGF* protein was detected in cultured human MC with immunoperoxidase staining using anti-VPF/*VEGF* antibody. TPA increased VPF/*VEGF* protein levels as well as those of VPF/*VEGF* mRNA in cultured human MC.(ABSTRACT TRUNCATED AT 250 WORDS)

2/3,AB/22 (Item 22 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07632586 93145946 PMID: 7678805

Synthesis and assembly of functionally active human vascular endothelial growth factor homodimers in insect cells.

Fiebich B L; Jager B; Schollmann C; Weindel K; Wilting J; Kochs G; Marme D; Hug H; Weich H A

Institute of Molecular Cell Biology, University of Freiburg, Federal Republic of Germany.

European journal of biochemistry / FEBS (GERMANY)

Jan 15 1993, 211 (1-2) p19-26, ISSN 0014-2956
Journal Code: 0107600
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Vascular endothelial growth factor (*VEGF*) is an angiogenic growth factor with a target-cell specificity highly restricted to vascular endothelial cells. Recombinant baculovirus were constructed for the production of two different forms of the human *VEGF* protein in insect cells. VEGF165 and VEGF121 proteins produced by Sf158 cells underwent a similar processing compared with mammalian cells, including efficient glycosylation, formation of a *disulfide*-linked dimer and secretion into the media. Only one of these forms, VEGF165 had a high affinity for heparin and this characteristic was used to purify this form to homogenicity by a two-step heparin-affinity chromatography. The biological activity of the purified 43-kDa homodimer was demonstrated by high-affinity binding to *VEGF* receptors, and by the induction of DNA synthesis in vascular endothelial cells. A positive angiogenic activity in vivo was demonstrated by the day-13 chorioallantoic-membrane assay. The mitogenic potency of VEGF121 for human umbilical vein endothelial cells was very similar compared to VEGF165. These results demonstrate that an angiogenic growth factor whose normal processing requires glycosylation and *disulfide* -bridge formation can be efficiently expressed in high concentration (up to 5 micrograms/ml) in insects cells.

2/3,AB/23 (Item 23 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

06750726 91060546 PMID: 2246236
Characterization of the receptors for vascular endothelial growth factor. Vaisman N; Gospodarowicz D; Neufeld G
Department of Biology, Israel Institute of Technology, Technion City, Haifa.
Journal of biological chemistry (UNITED STATES) Nov 15 1990, 265 (32) p19461-6, ISSN 0021-9258
Journal Code: 2985121R
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Vascular endothelial growth factor (*vEGF*) is a recently discovered mitogen for endothelial cells. It is also a potent angiogenic factor. We have characterized the *vEGF* receptors of endothelial cells using both binding and cross-linking techniques. Scatchard analysis of equilibrium binding experiments revealed two types of high-affinity binding sites on the cell surfaces of bovine endothelial cells. One of the sites has a dissociation constant of 10(-12) M and is present at a density of 3 x 10(3) receptors/cell. The other has a dissociation constant of 10(-11) M, with 4 x 10(4) receptors/cell. A high molecular weight complex containing 125I- *vEGF* is formed when 125I-*vEGF* is cross-linked to bovine endothelial cells. This complex has an apparent molecular mass of 225 kDa. Two other faintly labeled complexes with apparent molecular masses of 170 and 195 kDa also are detected. Reduction in the presence of dithiothreitol causes a substantial increase in the labeling intensity of the 170- and 195-kDa complexes, suggesting that these complexes are derived from the 225-kDa complex by reduction of *disulfide* bonds. The labeling of the *vEGF* receptors was inhibited by an excess of unlabeled *vEGF* but not by high concentrations of several other growth factors. Suramin and protamine, as well as several species of lectins, inhibited the binding. The expression of functional *vEGF* receptors was inhibited when the cells were preincubated with tunicamycin, indicating that glycosylation of the receptor is important for the expression of functional *vEGF* receptors. Pretreatment with swainsonine on the other hand, did not prevent formation of functional receptors. However, the mass of the 225-kDa complex is decreased by 20 kDa when 125I-*vEGF* is cross-linked to swainsonine-treated endothelial cells.

2/3,AB/24 (Item 1 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

08159715 Genuine Article#: 252ZL Number of References: 39 Title: Biosynthesis of vascular endothelial growth factor-D involves proteolytic processing which generates non-covalent homodimers (ABSTRACT AVAILABLE)
Author(s): Stacker SA (REPRINT) ; Stenvers K; Caesar C; Vitali A; Domagala T; Nice E; Roufail S; Simpson RJ; Moritz R; Karpanen T; Alitalo K; Achen MG
Corporate Source: ROYAL MELBOURNE HOSP,LUDWIG INST CANC RES, POB 2008/PARKVILLE/VIC 3050/AUSTRALIA/ (REPRINT); LUDWIG INST CANC RES,JOINT PROT STRUCT LAB/PARKVILLE/VIC 3050/AUSTRALIA/; ROYAL MELBOURNE HOSP,WALTER & ELIZA HALL INST MED RES/PARKVILLE/VIC 3050/AUSTRALIA/; UNIV HELSINKI,HAARTMAN INST, MOL CANC BIOL LAB/SF-00014 HELSINKI//FINLAND/
Journal: JOURNAL OF BIOLOGICAL CHEMISTRY, 1999, V274, N45 (NOV 5), P 32127-32136
ISSN: 0021-9258 Publication date: 19991105
Publisher: AMER SOC BIOCHEMISTRY MOLECULAR

BIOLOGY INC, 9650 ROCKVILLE PIKE, BETHESDA, MD 20814
Language: English Document Type: ARTICLE
Abstract: Vascular endothelial growth factor-D (*VEGF*-D) binds and activates the endothelial cell tyrosine kinase receptors *VEGF* receptor-2 (VEGFR-2) and *VEGF* receptor-3 (VEGFR-3), is mitogenic for endothelial cells, and shares structural homology and receptor specificity with *VEGF*-C, The primary translation product of *VEGF*-D has long N- and C-terminal polypeptide extensions in addition to a central *VEGF* homology domain (VHD). The VHD of *VEGF*-D is sufficient to bind and activate VEGFR-2 and VEGFR-3. Here we report that *VEGF*-D is proteolytically processed to release the VHD. Studies in 293EBNA cells demonstrated that *VEGF* -D undergoes Nand C-terminal cleavage events to produce numerous secreted polypeptides including a fully processed form of M-r similar to 21,000 consisting only of the VHD, which is predominantly a non-covalent dimer. Biosensor analysis demonstrated that the VHD has similar to 290- and similar to 40-fold greater affinity for VEGFR-2 and VEGFR-3, respectively, compared with unprocessed *VEGF*-D. In situ hybridization demonstrated that embryonic lung is a major site of expression of the *VEGF*-D gene. Processed forms of *VEGF*-D were detected in embryonic lung indicating that *VEGF*-D is proteolytically processed in vivo.

2/3,AB/25 (Item 2 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
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06693867 Genuine Article#: ZL064 Number of References: 53 Title: The alpha-helical domain near the amino terminus is essential for dimerization of vascular endothelial growth factor (ABSTRACT AVAILABLE)
Author(s): Siemeister G (REPRINT) ; Marme D; MartinyBaron G Corporate Source: INST MOL MED,TUMOR BIOL CTR, BREISACHER STR 117/D-79106 FREIBURG//GERMANY/ (REPRINT)
Journal: JOURNAL OF BIOLOGICAL CHEMISTRY, 1998, V273, N18 (MAY 1), P 11115-11120
ISSN: 0021-9258 Publication date: 19980501
Publisher: AMER SOC BIOCHEMISTRY MOLECULAR BIOLOGY INC, 9650 ROCKVILLE PIKE, BETHESDA, MD 20814
Language: English Document Type: ARTICLE
Abstract: Vascular endothelial growth factor (*VEGF*) is an endothelial cell-specific mitogen and a key mediator of aberrant endothelial cell proliferation and vascular permeability in a variety of human pathological situations such as tumor angiogenesis, diabetic retinopathy, or psoriasis, By amino-terminal deletion analysis and by site-directed mutagenesis we have identified a new domain within the amino-terminal alpha-helix that is essential for dimerization of *VEGF*. *VEGF*(121) variants containing amino acids 8 to 121 or 14 to 121, respectively, either expressed in Escherichia coil and refolded in vitro, or expressed in Chinese hamster ovary cells, were in a dimeric conformation and showed full binding activity to *VEGF* receptors and stimulation of endothelial cell proliferation as compared with wild-type *VEGF*, In contrast, a *VEGF*(121) variant covering amino acids 18 to 121, as well as a variant in which the hydrophobic amino acids Val(14), Val(15), Phe(17), and Met(18) within the amphipathic alpha-helix near the amino terminus were replaced by serine, failed to form biological active *VEGF* dimers, From these data we conclude that a domain between amino acids His(12) and Asp(19) within the aminoterminal alpha-helix is essential for formation of *VEGF* dimers, and we propose hydrophobic interactions between *VEGF* monomers to stabilize or favor dimerization.

2/3,AB/26 (Item 3 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
(c) 2002 Inst for Sci Info. All rts. reserv.

06164461 Genuine Article#: XY970 Number of References: 53 Title: Genomic organization of human and mouse genes for vascular endothelial growth factor C (ABSTRACT AVAILABLE)
Author(s): Chilov D; Kukk E; Taira S; Jeltsch M; Kaukonen J; Palotie A; Joukov V; Alitalo K (REPRINT)
Corporate Source: UNIV HELSINKI,HAARTMAN INST, MOL CANC BIOL LAB, PL21 HAARTMANINKATU 3/FIN-00014 HELSINKI//FINLAND/ (REPRINT); UNIV HELSINKI,HAARTMAN INST, MOL CANC BIOL LAB/FIN-00014 HELSINKI//FINLAND/
Journal: JOURNAL OF BIOLOGICAL CHEMISTRY, 1997, V272, N40 (OCT 3), P 25176-25183
ISSN: 0021-9258 Publication date: 19971003
Publisher: AMER SOC BIOCHEMISTRY MOLECULAR BIOLOGY INC, 9650 ROCKVILLE PIKE, BETHESDA, MD 20814
Language: English Document Type: ARTICLE
Abstract: We report here the cloning and characterization of human and mouse genes for vascular endothelial growth factor C (*VEGF*-C), a newly isolated member of the vascular endothelial growth factor/platelet-derived growth factor (*VEGF*/PDGF) family, Both *VEGF*-C genes comprise over 40 kilobase pairs of genomic DNA and consist of seven exons, all containing coding sequences, The *VEGF* homology domain of *VEGF*-C is encoded by exons 3 and 4, Exons 5 and 7 encode cysteine-rich motifs of the type C6C10CRC, and exon 6 encodes additional C10CXCXC motifs typical of a silk protein, A putative alternatively spliced rare RNA form lacking exon 4 was identified in

human fibrosarcoma cells, and a major transcription start site was located in the human *VEGF*-C gene 523 base pairs upstream of the translation initiation codon, The upstream promoter sequences contain conserved putative binding sites for Sp-1, AP-2, and NF-kappa B transcription factors but no TATA box, and they show promoter activity when transfected into cells, The *VEGF*-C gene structure is thus assembled from exons encoding propeptides and distinct cysteine-rich domains in addition to the *VEGF* homology domain, and it shows both similarities and distinct differences in comparison with other members of the *VEGF*/PDGF gene family.

2/3,AB/27 (Item 4 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
(c) 2002 Inst for Sci Info. All rts. reserv.

05952763 Genuine Article#: XJ990 Number of References: 54 Title: Proteolytic processing regulates receptor specificity and activity of *VEGF*-C (ABSTRACT AVAILABLE)
Author(s): Joukov V; Sorsa T; Kumar V; Jeltsch M; ClaessonWelsh L; Cao YH; Saksela O; Kalkkinen N; Alitalo K (REPRINT)
Corporate Source: UNIV HELSINKI,MOL CANC BIOL LAB, PL21 HAARTMANINKATU 3/FIN-00014 HELSINKI//FINLAND/ (REPRINT); UNIV HELSINKI,MOL CANC BIOL LAB/FIN-00014 HELSINKI//FINLAND/; UNIV HELSINKI,HAARTMAN INST, DEPT VIROL/FIN-00014 HELSINKI//FINLAND/; UNIV HELSINKI,INST BIOTECHNOL/FIN-00014 HELSINKI//FINLAND/; KAROLINSKA INST,DEPT CELL & MOL BIOL/S-17177 STOCKHOLM//SWEDEN/; LUDWIG INST CANC RES,/S-75124 UPPSALA//SWEDEN/
Journal: EMBO JOURNAL, 1997, V16, N13 (JUL 1), P3898-3911
ISSN: 0261-4189 Publication date: 19970701
Publisher: OXFORD UNIV PRESS, GREAT CLARENDON ST, OXFORD, ENGLAND OX2 6DP Language: English
Document Type: ARTICLE
Abstract: The recently identified vascular endothelial growth factor C (*VEGF*-C) belongs to the platelet-derived growth factor (PDGF)/ *VEGF* family of growth factors and is a ligand for the endothelial-specific receptor tyrosine kinases VEGFR-3 and VEGFR-2. The *VEGF* homology domain spans only about one-third of the cysteine-rich *VEGF*-C precursor. Here we have analysed the role of post-translational processing in *VEGF*-C secretion and function, as well as the structure of the mature *VEGF*-C. The stepwise proteolytic processing of *VEGF*-C generated several *VEGF*-C forms with increased activity towards VEGFR-3, but only the fully processed *VEGF*-C could activate VEGFR-2. Recombinant 'mature' *VEGF*-C made in yeast bound VEGFR-3 (K-D = 135 pM) and VEGFR-2 (K-D = 410 pM) and activated these receptors. Like *VEGF*, mature *VEGF*-C increased vascular permeability, as well as the migration and proliferation of endothelial cells, Unlike other members of the PDGF/*VEGF* family, mature *VEGF*-C formed mostly non-covalent homodimers. These data implicate proteolytic processing as a regulator of *VEGF*-C activity, and reveal novel structure-function relationships in the PDGF/*VEGF* family.

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98927 VASCULAR/TI
S3 0 S VEGF AND (VEGF OR VASCULAR)/TI
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? s s4 and (cysteine or cystine)
11507 S4
82029 CYSTEINE
9994 CYSTINE
S5 110 S4 AND (CYSTEINE OR CYSTINE)
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...examined 50 records (50)
...examined 50 records (100)
...completed examining records
S6 71 RD (unique items)
? s s6 not s2
71 S6
27 S2
S7 65 S6 NOT S2
? t s7/3,ab/all

7/3,AB/1 (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

13101927 21909317 PMID: 11912159
Nuclear factor kappaB dependency of platelet-activating factor-induced angiogenesis.
Ko Hyun-Mi; Seo Kook Heon; Han Su-Ji; Ahn Kyu Youn; Choi Il-Hwan; Koh Gou Young; Lee Hern-Ku; Ra Myung Suk; Im Suhn-Young
Department of Biological Sciences, The Institute of Basic Sciences, Research Institute of Medical Science, Chonnam National University, Kwangju 500-757, Japan.

Cancer research (United States) Mar 15 2002, 62 (6) p1809-14, ISSN 0008-5472 Journal Code: 2984705R
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
This study investigated the mechanisms of platelet-activating factor (PAF)-induced angiogenesis in a mouse model of Matrigel implantation. PAF induced a dose- and time-dependent angiogenic response. Inhibitors of nuclear factor (NF) kappaB expression or action, including antisense oligonucleotides to the p65 subunit of NFkappaB (p65 antisense) and antioxidants such as alpha-tocopherol and N-acetyl-L-*cysteine* , significantly reduced PAF-induced angiogenesis. In human umbilical vein endothelial cells, PAF-induced mRNA expression and protein synthesis of various NFkappaB-dependent angiogenic factors, such as tumor necrosis factor-alpha, interleukin-1alpha, basic fibroblast growth factor, and vascular endothelial growth factor (*VEGF*). The PAF-induced expression of the above mentioned factors was inhibited by p65 antisense or antioxidants. A significant inhibition of the angiogenic effect of PAF was achieved by anti-*VEGF* antibodies or soluble *VEGF* receptors such as KDR and flt-1 but not by antibodies against tumor necrosis factor-alpha, interleukin-1alpha, or basic fibroblast growth factor. These data indicate that PAF enhances angiogenesis through inducing NFkappaB activation, which in turn promotes the production of angiogenic factors such as *VEGF*.

7/3,AB/2 (Item 2 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

12723067 21605585 PMID: 11740818
Novel therapies targeting the myeloma cell and its bone marrow microenvironment.
Hideshima T; Chauhan D; Podar K; Schlossman R L; Richardson P; Anderson K C
Department of Adult Oncology, Jerome Lipper Multiple Myeloma Center, Dana-Farber Cancer Institute, 44 Binney Street, Boston, MA 02115, USA. Seminars in oncology (United States) Dec 2001, 28 (6) p607-12, ISSN 0093-7754 Journal Code: 0420432
Contract/Grant No.: CA 50947; CA; NCI; CA 78378; CA; NCI Document type: Journal Article; Review; Review, Tutorial Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Novel therapies in multiple myeloma (MM) target not only the tumor cell but also the bone marrow (BM) microenvironment. Thalidomide (Thal), as well as derivative immunomodulatory drugs (IMiDs), directly induce apoptosis or G1 growth arrest in MM cell lines and patient's MM cells which are resistant to melphalan (Mel), doxorubicin (Dox), and dexamethasone (Dex). Although Thal and IMiDs do not alter adhesion of MM cells to bone marrow stromal cells (BMSCs), they inhibit the upregulation of interleukin-6 (IL-6) and vascular endothelial growth factor (*VEGF*) secretion triggered by the binding of MM cells to BMSCs. Proteasome inhibitors represent another potential anticancer therapy targeting the MM cell and the BM microenvironment. The proteasome inhibitor PS-341 directly inhibits proliferation and induces apoptosis in both human MM cell lines and freshly isolated patient's MM cells which are resistant to Mel, Dox, and Dex. PS-341 inhibits p44/42 mitogen-activated protein kinase (MAPK) growth signaling triggered by IL-6 and induces apoptosis, despite induction of p21 and p27, in p53 wild-type and p53 mutant MM cells. PS-341 adds to the anti-MM activity of dexamethasone and overcomes IL-6-mediated protection against dexamethasone-induced apoptosis. PS-341 blocks the paracrine growth of human MM cells by decreasing their adherence to BMSCs and related NF-kappaB-dependent induction of IL-6 secretion in BMSCs. Moreover, proliferation and MAPK growth signaling of those residual adherent MM cells is also inhibited. Tumor necrosis factor-alpha (TNF-alpha), which is produced by some MM cells, induces only low-level MM proliferation and MAPK activation in MM cells, but markedly upregulates IL-6 secretion from BMSCs and upregulates expression of adhesion molecules (VLA-4 and LFA-1) on MM cells and their receptors (VCAM-1 and ICAM-1) on BMSCs, with resultant increased binding of MM cells to BMSCs. Inhibition of TNF-alpha-induced NF-kappaB activation with PS-341 inhibits both the upregulation of these molecules on MM cells and BMSCs and the resultant increased adhesion. Therefore, inhibiting TNF-alpha and its sequelae may be useful treatment strategies in MM. Our data show that *VEGF* causes proliferation and enhances migration of MM as well as plasma cell leukemia (PCL) cells. *VEGF* induced twofold activation of cell migration in MM cells and more than 100-fold activation of cell migration in PCL cells, suggesting an important role of *VEGF* in the progression of MM to PCL. These data indicate that *VEGF* plays a pivotal role not only in neoangiogenesis in MM BM but also in proliferation and migration of tumor cells. Copyright 2001 by W.B. Saunders Company.

7/3,AB/3 (Item 3 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

12632218 21575840 PMID: 11719447
Inhibition of angiogenesis-driven Kaposi's sarcoma tumor growth in nude mice by oral N-acetylcysteine.
Albini A; Morini M; D'Agostini F; Ferrari N; Campelli F; Arena G; Noonan D M; Pesce C; De Flora S

National Institute for Cancer Research (IST), c/o Advanced Biotechnology Center, Largo R. Benzi 10, I-16132 Genoa, Italy. albini@vega.cba.unige.it Cancer research (United States) Nov 15 2001, 61 (22) p8171-8, ISSN 0008-5472 Journal Code: 2984705R
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The thiol N-acetyl-L-*cysteine* (NAC), an analogue and precursor of reduced glutathione, has cancer chemopreventive properties attributable to its nucleophilicity, antioxidant activity, and a variety of other mechanisms. We demonstrated recently that NAC has anti-invasive, antimetastatic, and antiangiogenic effects in in vitro and in vivo test systems. In the present study, s.c. transplantation of KS-Imm cells in (CD-1)BR nude mice resulted in the local growth of Kaposi's sarcoma, a highly vascularized human tumor. The daily administration of NAC with drinking water, initiated after the tumor mass had become established and detectable, produced a sharp inhibition of tumor growth, with regression of tumors in half of the treated mice along with a markedly prolonged median survival time. The production of vascular endothelial growth factor (*VEGF*) and certain proliferation markers (proliferating cell nuclear antigen and Ki-67) were significantly lower in Kaposi's sarcomas from NAC-treated mice than from control mice. Treatment of KS-Imm cells with NAC in vitro resulted in a dose-dependent inhibition of chemotaxis and invasion through inhibition of gelatinase-A (matrix metalloproteinase-2, MMP-2) activity without altering MMP-2 or MMP-9 mRNA levels. NAC also significantly inhibited *VEGF* production but did not affect proliferation markers in vitro. Reverse transcription-PCR analysis indicated that total *VEGF* mRNAs were reduced by 10 mM NAC. Taken together, these findings provide evidence that NAC, the safety of which even at high doses has been established in almost 40 years of clinical use, in addition to its chemopreventive action, has a strong antiangiogenic potential that could be exploited for preventing cancer progression as well as used in cancer adjuvant therapy.

7/3,AB/4 (Item 4 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

12626996 21570249 PMID: 11571295
Regulation of vascular endothelial growth factor expression by advanced glycation end products.
Treins C; Giorgetti-Peraldi S; Murdaca J; Van Obberghen E INSERM U145, IFR 50, Faculte de Medecine, Avenue de Valombrose, Nice 06107, Cedex 2, France.
Journal of biological chemistry (United States) Nov 23 2001, 276 (47) p43836-41, ISSN 0021-9258 Journal Code: 2985121R
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Advanced glycation end products (AGEs) are generated during long term diabetes and are correlated with the development of diabetic complications, such as retinopathy. Diabetic retinopathy is characterized by an increased retinal neovascularization due to the action of the angiogenic factor, vascular endothelial growth factor (*VEGF*). In this report, we show that injection of insulin and glycated albumin (Alb-AGE) to mice increases *VEGF* mRNA expression in eyes. Insulin and Alb-AGE stimulate *VEGF* mRNA and protein expression in retinal epithelial cells (ARPE-19). Alb-AGE-induced *VEGF* expression is not modulated by the use of antioxidants, N-acetyl-l-*cysteine* or pyrrolidinedithiocarbamat e, or by an inhibitor of phosphatidylinositol 3-kinase (PI3K), wortmannin. However, using an inhibitor of ERK activation, U0126, we show that Alb-AGE stimulates *VEGF* expression through an ERK-dependent pathway. Accordingly, we found that Alb-AGE activated mitogen-activate protein kinase, ERK1/2, JNK1/2, but not p38, and that Alb-AGE did not activate PI3K and PKB. Moreover, Alb-AGE activated the transcription factor, hypoxia inducible factor-1 (HIF-1) DNA binding activity. This activation is mediated by an increase in accumulation of the HIF-1alpha protein through an ERK-dependent pathway. Thus, stimulation of *VEGF* expression by Alb-AGE, through the activation of HIF-1, could play an important role in the development of diabetic retinopathy.

7/3,AB/5 (Item 5 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

12598334 21543944 PMID: 11688957
HIV-Tat dependent chemotaxis and invasion, key aspects of tat mediated pathogenesis.
Vene R; Benelli R; Noonan D M; Albini A
Advanced Biotechnology Center Genoa, Italy.
Clinical & experimental metastasis (Netherlands) 2000, 18 (7) p533-8 , ISSN 0262-0898 Journal Code: 8409970
Document type: Journal Article; Review; Review, Tutorial
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Extracellular Tat acts as a pleiotropic molecule inducing several biological effects on different target cells. Tat stimulates the chemotaxis of numerous cell types and it appears to have oncogenic activities,

including acting as a co-factor for Kaposi's sarcoma. The Tat protein has been shown to bind integrins through an RGD amino acid motif. Tat is an angiogenic factor able to induce the migration and invasion of endothelial and KS cells through the interaction of its basic domain with the *VEGF* receptor VEGFR2 (Flk-1/KDR). We have also found that Tat is able to mimic chemokines, activating monocyte migration through the chemokine like' *cysteine* -core domain. Tat is a chemoattractant for dendritic cells, and both the RGD and basic domains appear to be involved in this response. In a recent study we demonstrated that Tat is chemotactic for PMN and induces Ca2+ mobilization in vitro. Experiments using synthetic peptides showed that Tat activities on PMN are mediated by the chemokine like' region. Finally Tat is also able to induce B cell chemotaxis, while its activity on helper T cells has not yet been clarified. Here we review data on Tat-dependent chemotaxis and discuss the possible implications in Tat mediated pathogenesis.

7/3,AB/6 (Item 6 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

12585406 21523943 PMID: 11517223
The von Hippel-Lindau protein interacts with heteronuclear ribonucleoprotein a2 and regulates its expression.
Pioli P A; Rigby W F
Department of Medicine, Dartmouth Medical School, Lebanon, New Hampshire 03756, USA.
Journal of biological chemistry (United States) Oct 26 2001, 276 (43) p40346-52, ISSN 0021-9258 Journal Code: 2985121R
Contract/Grant No.: R01 AI34928; AI; NIAID; T32 AI 10736 3; AI; NIAID Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The product of the von Hippel-Lindau (VHL) tumor suppressor gene, pVHL, functions as a ubiquitin-protein isopeptide ligase in regulating HIF-1 protein turnover, thus accounting for the increased transcription of hypoxia-inducible genes that accompanies VHL mutations. The increased vascular endothelial growth factor mRNA stability in cells lacking pVHL has been hypothesized to be due to a similar regulation of an RNA-binding protein. We report the expression of the GLUT-1 3'-untranslated region RNA-binding protein, heteronuclear ribonucleoprotein (hnRNP) A2, is specifically increased in pVHL-deficient cell lines. Enhanced hnRNP A2 expression was apparent in all cell fractions, including polysomes, where a similar modest effect on hnRNP L (a GLUT-1 and *VEGF* 3'-untranslated region-binding protein), was seen. Steady state levels of hnRNP A2 mRNA were unaffected. Regulation of hnRNP A2 levels correlated with the ability of pVHL to bind elongin C. Proteasome inhibition of cells expressing wild type pVHL selectively increased cytoplasmic hnRNP A2 levels to that seen in pVHL-deficient cells. Finally, an in vivo interaction between pVHL and hnRNP A2 was demonstrated in both the nucleus and the cytoplasm. Collectively, these data indicate that hnRNP A2 expression is regulated by pVHL in a manner that is dependent on elongin C interactions as well as functioning proteasomes.

7/3,AB/7 (Item 7 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

12534178 21397851 PMID: 11506896
Induction of vascular endothelial growth factor by nitric oxide in cultured human articular chondrocytes.
Turpaev K; Litvinov D; Dubovaya V; Panasyuk A; Ivanov D; Prassolov V Engelhardt Institute of Molecular Biology, ul. Vavilova 32, Russian Academy of Sciences, 117984, Moscow, Russia. turpaev@imb.ac.ru Biochimie (France) Jun 2001, 83 (6) p515-22, ISSN 0300-9084 Journal Code: 1264604
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
We investigated the role of nitric oxide (NO) in the control of vascular endothelial growth factor A (*VEGF*) gene expression in cultured human articular chondrocytes. Cell treatment with the NO-generating compound nitrosoglutathione (GSNO) caused a significant accumulation of 4.4 kb *VEGF* mRNA, a major *VEGF* mRNA isoform expressing in chondrocytes. This is the first demonstration that NO can induce *VEGF* mRNA expression in chondrocytes. *VEGF* mRNA level was not affected in cells exposed to dibutyryl cGMP, a non-hydrolyzable analog of cGMP, suggesting that the cGMP system is not involved in NO-dependent transcriptional activation of *VEGF* gene. The GSNO-stimulated induction of *VEGF* mRNA was slightly attenuated by MAP protein kinase inhibitors PD98058 and SB203580, but was completely blocked in cells incubated with GSNO in the presence of catalase and superoxide dismutase, enzymes scavenging reactive oxygen species (ROS), or in the presence of thiol-containing antioxidants, N-acetyl *cysteine* and reduced glutathione. These results suggest that in articular chondrocytes the GSNO-induced *VEGF* gene transcriptional activation is dependent on endogenous ROS production and oxidative thiol modifications.

7/3,AB/8 (Item 8 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

11298097 21338249 PMID: 11444882
Gene expression profiling of an arteriogenic impotence model. Lin C S; Ho H C; Gholami S; Chen K C; Jad A; Lue T F
Knuppe Molecular Urology Laboratory, School of Medicine, University of California at San Francisco, San Francisco, CA 94143-1695, USA. clin@urol.ucsf.edu
Biochemical and biophysical research communications (United States) Jul 13 2001, 285 (2) p565-9, ISSN 0006-291X Journal Code: 0372516 Contract/Grant No.: 2R01-DK-45370; DK; NIDDK
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Penile arterial insufficiency is one of the most common causes of ED. We have established a traumatic arteriogenic insufficiency rat model by the ligation of the pudendal arteries. To simulate both acute and chronic traumatic injuries, five ligation periods (6 h, 3 days, 7 days, 3 weeks, and 6 weeks) were chosen. By electrostimulation of the cavernous nerve, the intracavernous pressure was determined to be between 20 and 40-cm H(2)O for the ligated rats compared to around 100-cm H(2)O for the control rats. The erectile tissue in the corpus cavernosum of these rats was then subjected to microarray analysis, in which an array that contains cDNA fragments representing 1176 rat genes was used. The results demonstrated that normal rat corpus cavernosum expressed approximately 200 genes at detectable levels and that ligation produced differential expression of approximately 25 genes, depending on the duration of ligation. The most highly ligation-induced gene was apolipoprotein D (ApoD), with peak expression in the 3- and 7-day ligated rats. Three of the insulin-like growth factor binding proteins (IGFBP-1, 3, and 5) were upregulated in all ligated rats. IGFBP-6, which was one of the most highly expressed genes in the normal corpus cavernosum, was down-regulated in all ligated rats. *Cysteine* proteases of the cathepsin family were also differentially expressed between control and ligated rats, with cathepsin K being down-regulated most. A few genes were upregulated only in the 6-week ligated rats, including angiotensin-converting enzyme. Finally, *VEGF* , whose induction has been identified in many other ischemic tissues, was not induced in corpus cavernous tissue of ligated rats. Copyright 2001 Academic Press.

7/3,AB/9 (Item 9 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

11277876 21316593 PMID: 11424091
Role of transcription factor Ets-1 in the apoptosis of human vascular endothelial cells.
Teruyama K; Abe M; Nakano T; Iwasaka-Yagi C; Takahashi S; Yamada S; Sato Y
Department of Vascular Biology, Institute of Development, Aging and Cancer, Tohoku University, Sendai, Japan.
Journal of cellular physiology (United States) Aug 2001, 188 (2) p243-52, ISSN 0021-9541 Journal Code: 0050222
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Transcription factor Ets-1 is induced in endothelial cells (ECs) by angiogenic factors, and promotes angiogenesis by inducing angiogenesis-related genes such as MMPs and integrin beta3. Here, we examined the effect of Ets-1 on apoptosis in ECs. Overexpression of Ets-1 in human umbilical vein endothelial cells (HUVECs) induced apoptosis under the serum-deprived condition. *VEGF* inhibited apoptosis and augmented the DNA binding of Ets-1 in HUVECs. The inhibition of transcriptional activity of endogenous Ets-1 by a dominant negative molecule intensified the anti-apoptotic effect of *VEGF* . Caspase inhibitors blocked apoptosis of HUVECs induced by Ets-1. DNA array analysis showed that Ets-1 up-regulated pro-apoptotic genes such as Bid, cytochrome p450, caspase-4, p27, and p21 more than 2 fold, and down-regualted anti-apoptotic genes such as DAD-1, AXL, Cox-2, IAP-2, and MDM-2 less than 0.5 fold in HUVECs. These results indicate that Ets-1 itself is pro-apoptotic to ECs by modulating the expression of apoptosis-related genes. Copyright 2001 Wiley-Liss, Inc.

7/3,AB/10 (Item 10 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

11217220 21241096 PMID: 11342715
Folding screening assayed by proteolysis: application to various *cystine* deletion mutants of vascular endothelial growth factor. Heiring C; Muller Y A
Forschungsgruppe Kristallographie, Max-Delbruck-Center for Molecular Medicine, Robert-Rossle-Strasse 10, D-13092 Berlin, Germany.
Protein engineering (England) Mar 2001, 14 (3) p183-8, ISSN 0269-2139 Journal Code: 8801484
Document type: Evaluation Studies; Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The production of recombinant proteins in Escherichia coli often leads to the formation of inclusion bodies. Although this has a number of advantages, a major disadvantage is the need to develop folding protocols for

the renaturing of the proteins. However, the systematic screening of folding conditions is often hampered by the lack of convenient assays to detect correctly folded proteins. To address this problem we present a simple protocol, which combines folding screens and limited proteolysis to rapidly assess and optimize folding conditions. The efficacy of this method, termed FSAP (folding screening assayed by proteolysis), is demonstrated by the large-scale folding, purification and crystallization of various *cystine* deletion mutants of the *cystine* knot family member: vascular endothelial growth factor (*VEGF*). These mutants are particularly difficult to fold as the *cystine* knot is believed to make major contributions to the stability of the protein and this family of proteins lacks extensive hydrophobic core regions.

7/3,AB/11 (Item 11 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

11206175 21226163 PMID: 11327725
Cysteine -rich and basic domain HIV-1 Tat peptides inhibit angiogenesis and induce endothelial cell apoptosis.
Jia H; Lohr M; Jezequel S; Davis D; Shaikh S; Selwood D; Zachary I Department of Medicine, Ark Therapeutics Limited, London, WC1E 6JJ, United Kingdom.
Biochemical and biophysical research communications (United States) May 4 2001, 283 (2) p469-79, ISSN 0006-291X Journal Code: 0372516 Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Previous findings suggest that both the Tat polypeptide encoded by HIV-1 and Tat-derived peptides can induce angiogenesis via activation of the KDR receptor for Vascular Endothelial Growth Factor (*VEGF*). We identified 20 amino acids and 12 amino acid peptides corresponding to the *cysteine*-rich and basic domains of HIV-1 Tat which inhibited (125)I-*VEGF* (165) binding to KDR and neuropilin-1 (NP-1) receptors in endothelial cells. *Cysteine*-rich and basic Tat peptides inhibited *VEGF*-induced ERK activation and mitogenesis in endothelial cells, and inhibited angiogenesis in vitro at concentrations similar to those which inhibited *VEGF* receptor binding. These peptides also inhibited proliferation, angiogenesis, and ERK activation induced by basic fibroblast growth factor with similar potency and efficacy. Surprisingly, we found that both *cysteine* -rich and basic domain Tat peptides strikingly induced apoptosis in endothelial cells, independent of their effects on *VEGF* and bFGF. Furthermore, we found no evidence for direct biological effects of recombinant Tat on *VEGF* receptor binding, ERK activation, endothelial cell survival, or mitogenesis. These findings demonstrate novel properties of Tat-derived peptides and indicate that their major effect in endothelial cells is apoptosis independent of specific inhibition of *VEGF* receptor activation.

7/3,AB/12 (Item 12 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

11179088 21192270 PMID: 11069911
The crystal structure of human placenta growth factor-1 (PlGF-1), an angiogenic protein, at 2.0 A resolution.
Iyer S; Leonidas D D; Swaminathan G J; Maglione D; Battisti M; Tucci M; Persico M G; Acharya K R
Department of Biology and Biochemistry, University of Bath, Claverton Down, Bath BA2 7AY, United Kingdom.
Journal of biological chemistry (United States) Apr 13 2001, 276 (15) p12153-61, ISSN 0021-9258 Journal Code: 2985121R
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The angiogenic molecule placenta growth factor (PlGF) is a member of the *cysteine* -knot family of growth factors. In this study, a mature isoform of the human PlGF protein, PlGF-1, was crystallized as a homodimer in the crystallographic asymmetric unit, and its crystal structure was elucidated at 2.0 A resolution. The overall structure of PlGF-1 is similar to that of vascular endothelial growth factor (*VEGF*) with which it shares 42% amino acid sequence identity. Based on structural and biochemical data, we have mapped several important residues on the PlGF-1 molecule that are involved in recognition of the fms-like tyrosine kinase receptor (Flt-1, also known as VEGFR-1). We propose a model for the association of PlGF-1 and Flt-1 domain 2 with precise shape complementarity, consider the relevance of this assembly for PlGF-1 signal transduction, and provide a structural basis for altered specificity of this molecule.

7/3,AB/13 (Item 13 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

11172217 21182309 PMID: 11285368
Time course of LPS-induced gene expression in a mouse model of genitourinary inflammation.
Saban M R; Hellmich H; Nguyen N B; Winston J; Hammond T G; Saban R Department of Physiology, University Oklahoma Health Sciences Center, Oklahoma

City, Oklahoma 73190, USA.
Physiological genomics (Online) (United States) Apr 2 2001, 5 (3) p147-60, ISSN 1094-8341 Journal Code: 100894125
Contract/Grant No.: DK-51392; DK; NIDDK; DK-55828-01; DK; NIDDK Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
In this study, self-organizing map (SOM) gene cluster techniques are applied to the analysis of cDNA microarray analysis of gene expression changes occurring in the early stages of genitourinary inflammation. We determined the time course of lipopolysaccharide (LPS)-induced gene expression in experimental cystitis. Mice were euthanized 0.5, 1, 4, and 24 h after LPS instillation into the urinary bladder, and gene expression was determined using four replicate Atlas mouse cDNA expression arrays containing 588 known genes at each time point. SOM gene cluster analysis, performed without preconditions, identified functionally significant gene clusters based on the kinetics of change in gene expression. Genes were classified as follows: 1) expressed at time 0; 2) early genes (peak expression between 0.5 and 1 h); and 3) late genes (peak expression between 4 and 24 h). One gene cluster maintained a constant level of expression during the entire time period studied. In contrast, LPS treatment downregulated the expression of some genes expressed at time 0, in a cluster including transcription factors, protooncogenes, apoptosis-related proteins (*cysteine* protease), intracellular kinases, and growth factors. Gene upregulation in response to LPS was observed as early as 0.5 h in a cluster including the interleukin-6 (IL-6) receptor, alpha- and beta-nerve growth factor (alpha- and beta-NGF), vascular endothelial growth factor receptor-1 (*VEGF* R1), C-C chemokine receptor, and P-selectin. Another tight cluster of genes with marked expression at 1 h after LPS and insignificant expression at all other time points studied included the protooncogenes c-Fos, Fos-B, Fra-2, Jun-B, Jun-D, and Egr-1. Almost all interleukin genes were upregulated as early as 1 h after stimulation with LPS. Nuclear factor-kappaB (NF-kappaB) pathway genes collected in a single cluster with a peak expression 4 h after LPS stimulation. In contrast, most of the interleukin receptors and chemokine receptors presented a late peak of expression 24 h after LPS coinciding with the peak of neutrophil infiltration into the bladder wall. Selected cDNA microarray observations were confirmed by RNase protection assay. In conclusion, the cDNA array experimental approach provided a global profile of gene expression changes in bladder tissue after stimulation with LPS. SOM techniques identified functionally significant gene clusters, providing a powerful technical basis for future analysis of mechanisms of bladder inflammation.

7/3,AB/14 (Item 14 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

11027218 21024577 PMID: 11148815
Regulation of connective tissue growth factor gene expression in retinal vascular endothelial cells by angiogenic growth factors.
Wunderlich K; Senn B C; Todesco L; Flammer J; Meyer P
Department of Ophthalmopathology, University Eye Clinic, 4012 Basel, Switzerland.
Graefe's archive for clinical and experimental ophthalmology = Albrecht von Graefes Archiv fur klinische und experimentelle Ophthalmologie (Germany) Nov 2000, 238 (11) p910-5, ISSN 0721-832X Journal Code: 8205248 Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
BACKGROUND: Connective tissue growth factor (CTGF) is a novel, *cysteine* -rich secreted protein, which is implicated in fibrotic disorders and atherosclerosis. To elucidate the role of CTGF in fibrovascular proliferative retinopathy, we investigated the regulation of CTGF gene expression in a cell line of retinal vascular endothelial cells (RVEC) stimulated with fetal calf serum (FCS) and angiogenic growth factors, including vascular endothelial growth factor (*VEGF*), basic fibroblast growth factor (bFGF), platelet-derived growth factor-BB (PDGF-BB), endothelial growth factor (EGF), transforming growth factor-beta 1 and -beta 3 (TGF-beta 1, TGF-beta 3), and insulin-like growth factor-I (IGF-I). METHODS: RVEC derived from Macaca mulatta (CRL-1780; ATCC) were stimulated with 10% FCS as well as with *VEGF*, bFGF, PDGF-BB, TGF-beta 1, TGF-beta 3, EGF, or IGF-I. Time-dependent CTGF gene expression was assessed by northern blot analysis. RESULTS: FCS, TGF-beta 1, TGF-beta 3, bFGF, and EGF induced an upregulation of CTGF gene expression in RVEC in a time-dependent manner. Highest expression was induced with TGF-beta 1. No response on CTGF gene expression could be detected to *VEGF*, PDGF-BB, or IGF-I. CONCLUSION: The present study demonstrates for the first time that CTGF mRNA is expressed at high levels in RVEC, and that the level of the temporal pattern of its expression is differentially regulated by angiogenic growth factors, indicating a significant role of CTGF in the pathological course of uncontrolled retinal angiogenesis.

7/3,AB/15 (Item 15 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10988406 20563797 PMID: 11112413
iNOS expression inhibits hypoxia-inducible factor-1 activity. Yin J H; Yang D I; Ku G; Hsu C Y
Department of Medicine, Tao-Yuan Armed Forces General Hospital, Taiwan. Biochemical and biophysical research communications (UNITED STATES) Dec 9 2000, 279 (1) p30-4, ISSN 0006-291X Journal Code: 0372516 Contract/Grant No.: 28995; PHS; 37230; PHS; 40162; PHS; + Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Hypoxia-inducible factor-1 (HIF-1) activates genes important in vascular function such as vascular endothelial growth factor (*VEGF*), erythropoietin (EPO), and inducible nitric oxide synthase (iNOS). iNOS catalyzes the synthesis of nitric oxide (NO), a free radical gas that mediates a number of cellular processes, including regulation of gene expression, vasodilatation, and neurotransmission. Here we demonstrate that iNOS expression inhibits HIF-1 activity under hypoxia in C6 glioma cells transfected with an iNOS gene and a *VEGF* promoter-driven luciferase gene. HIF-1 induction of *VEGF*-luciferase activity in C6 cell is also inhibited by sodium nitroprusside (SNP). Furthermore, pretreatment of C6 cells with N-acetyl-l-*cysteine* (NAC), an antioxidant, nullified the inhibitory effect of iNOS on HIF-1 binding. These results demonstrate that NO generated by iNOS expression inhibits HIF-1 activity in hypoxic C6 cells and suggest a negative feedback loop in the HIF-1 --> iNOS cascade. Copyright 2000 Academic Press.

7/3,AB/16 (Item 16 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10960588 20538754 PMID: 11085806
Effects of combinations of anti-rheumatic drugs on the production of vascular endothelial growth factor and basic fibroblast growth factor in cultured synoviocytes and patients with rheumatoid arthritis. Nagashima M; Wauke K; Hirano D; Ishigami S; Aono H; Takai M; Sasano M; Yoshino S
Department of Joint Disease and Rheumatism, Nippon Medical School, 1-1-5, Sendagi, Bunkyo-ku, Tokyo, Japan.
Rheumatology (Oxford, England) (ENGLAND) Nov 2000, 39 (11) p1255-62, ISSN 1462-0324 Journal Code: 100883501
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
OBJECTIVE: To examine whether different combinations of disease-modifying anti-rheumatic drugs (DMARDs), including bucillamine (BUC), gold sodium thiomalate (GST), methotrexate (MTX), salazosulphapyridine (SASP) and dexamethasone (DEX; a steroid), act by inhibiting the production of vascular endothelial growth factor (*VEGF*) and basic fibroblast growth factor (bFGF) in cultured synoviocytes, causing a decrease in their serum concentrations in patients with rheumatoid arthritis (RA). METHODS: The *VEGF* and bFGF concentrations in cultured synoviocytes and peripheral blood from patients with RA were measured by enzyme-linked immunosorbent assay and their serum concentrations were measured at two time points. RESULTS: BUC and GST inhibited *VEGF* production even when given alone, and a combination of BUC, GST and MTX with DEX also inhibited *VEGF* production. None of the DMARDs or DEX inhibited bFGF production when given alone, but a combination of SASP and GST inhibited the production of bFGF in cultured synoviocytes. Serum *VEGF* concentrations were significantly decreased 6 months after the commencement of medication compared with their concentrations before medication. CONCLUSION: Our results show that the effects of a combination of DEX with any two of BUC, GST, SASP and MTX on the production of *VEGF* and bFGF in cultured synoviocytes and on the serum concentrations of *VEGF* in patients with RA may be based on synergistic or additive effects of the drugs.

7/3,AB/17 (Item 17 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10931426 20488297 PMID: 11036826
Imbalance in production between vascular endothelial growth factor and endostatin in patients with rheumatoid arthritis.
Nagashima M; Asano G; Yoshino S
Department of Joint Disease and Rheumatism, Nippon Medical School, Tokyo, Japan. Nagashima Masakazu/rheum@nms.ac.jp
Journal of rheumatology (CANADA) Oct 2000, 27 (10) p2339-42, ISSN 0315-162X Journal Code: 7501984
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
OBJECTIVE: To clarify whether synovial cell proliferation indicates an imbalance in production between angiogenic growth factors and angiogenesis inhibitors in rheumatoid arthritis (RA), we investigated the production of basic fibroblast growth factor (b-FGF) and vascular endothelial growth factor (*VEGF*) as representative angiogenic growth factors and endostatin as a representative angiogenesis inhibitor. METHODS: The b-FGF, *VEGF* , and endostatin levels in 90 samples of peripheral blood (PB) and 15 samples of

joint fluid obtained from patients with RA and 30 samples of PB and 10 samples of joint fluid from patients without RA, including 20 patients with inflammatory arthritis without purulent arthritis, and 10 patients with osteoarthritis were measured by ELISA. *VEGF* and endostatin levels in blood samples from 22 patients with RA were measured at 2 points: before and 4 or 5 months after the commencement of medication. RESULTS: The b-FGF and *VEGF* levels in the PB and joint fluid samples from patients with RA were markedly elevated compared to samples from patients without RA. In contrast, endostatin levels in PB and joint fluid samples from patients with RA were almost the same as in the samples from patients without RA. *VEGF* levels in blood samples obtained 4 or 5 months after the commencement of medication (combination of prednisolone 5 mg/day and disease modifying antirheumatic drugs: either bucillamine 100 mg/day or salazosulfapyridine 1,000 mg/day) were significantly decreased from 27.1 +/- 8.5 pg/ml in samples obtained before commencement of medication to 18.1 +/- 16.2 pg/ml. Endostatin levels in the corresponding samples were significantly increased, from 31.5 +/- 7.0 to 57.1 +/- 22.8 pg/ml. CONCLUSION: Our results reveal significant differences in b-FGF and *VEGF* levels in PB and joint fluid samples, but no difference in endostatin levels, between patients with RA and those without RA, suggesting that angiogenesis in RA occurs as a result of an imbalance in production between angiogenic growth factors and angiogenesis inhibitors.

7/3,AB/18 (Item 18 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10891117 20442196 PMID: 10985890
Alterations in SPARC and *VEGF* immunoreactivity in epithelial ovarian cancer.
Paley P J; Goff B A; Gown A M; Greer B E; Sage E H
The Division of Gynecologic Oncology, University of Washington, Seattle, Washington 98195, USA.
Gynecologic oncology (UNITED STATES) Sep 2000, 78 (3 Pt 1) p336-41, ISSN 0090-8258 Journal Code: 0365304
Contract/Grant No.: GM40711; GM; NIGMS
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
OBJECTIVE: Secreted protein, acidic and rich in *cysteine* (SPARC), is a matricellular protein that modulates cell adhesion and growth. It is thought to play a decisive role in tissue remodeling and angiogenesis. Alterations in SPARC expression have been observed in a variety of solid tumors; however, no consistent pattern of deregulation has been characterized. Vascular endothelial growth factor (*VEGF*) has emerged as an important regulator of tumor neovascularization. Recent work has shown that SPARC modulates the mitogenic activity of *VEGF* in normal endothelium. While its role in malignant transformation remains elusive, SPARC may contribute to tumor propagation and invasion. This study examines the immunoreactivity of SPARC and *VEGF* associated with neoplastic transformation of the ovary. METHODS: Immunostaining for *VEGF* and SPARC protein was performed on 62 archival specimens. RESULTS: Fourteen normal ovaries and 48 ovarian carcinomas were evaluated. SPARC was detected in the stroma of 63% of ovarian carcinomas. In contrast, SPARC was observed in the stroma of only 29% of normal ovaries (P = 0.02). Furthermore, SPARC was limited in normal ovaries to premenopausal patients, juxtaposed either with vesiculated follicles or within the corpus luteum. *VEGF* was observed in 42% of ovarian carcinomas with immunoreactivity confined to tumor cells. The level of *VEGF* immunoreactivity was significantly higher in ovarian carcinoma compared to normal ovary epithelium (42 vs 7%, P = 0.02). CONCLUSIONS: Immunoreactivity of SPARC and *VEGF* is heightened in association with ovarian carcinoma, with a distinct distribution of SPARC in the stroma of neoplastic ovaries and *VEGF* within tumor cells. No obvious pattern of coincident SPARC and *VEGF* immunoreactivity was detected. These results indicate the possibility of an aberration in the interaction that has been described in normal endothelium between SPARC and *VEGF* in association with malignant transformation. Copyright 2000 Academic Press.

7/3,AB/19 (Item 19 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10780765 20318445 PMID: 10859480
Thrombospondin-1 induces endothelial cell apoptosis and inhibits angiogenesis by activating the caspase death pathway.
Nor J E; Mitra R S; Sutorik M M; Mooney D J; Castle V P; Polverini P J Department of Oral Medicine/Pathology/Oncology, University of Michigan School of Dentistry, Ann Arbor, MI, USA.
Journal of vascular research (SWITZERLAND) May-Jun 2000, 37 (3) p209-18, ISSN 1018-1172 Journal Code: 9206092
Contract/Grant No.: CA64416; CA; NCI; HL39926; HL; NHLBI Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Thrombospondin-1 (TSP1) is a potent natural inhibitor of angiogenesis. Although TSP1 has been reported to induce endothelial cell apoptosis in vitro and to

downregulate neovascularization in vivo, the molecular mechanisms that link these two processes have yet to be established. Here we report that TSP1 mediates endothelial cell apoptosis and inhibits angiogenesis in association with increased expression of Bax, decreased expression of Bcl-2, and processing of caspase-3 into smaller proapoptotic forms. The ability of TSP1 to induce both endothelial cell apoptosis in vitro and to suppress angiogenesis in vivo was blocked by the caspase-3 inhibitor z-DEVD-FMK. TSP1 also attenuated *VEGF* -mediated Bcl-2 expression in endothelial cells in vitro and angiogenesis in vivo. Furthermore, TSP1 induced endothelial cell apoptosis and inhibited neovascularization in sponge implants in SCID mice. We conclude that TSP1 induces endothelial cell apoptosis and inhibits neovascularization by altering the profile of survival gene expression and activating caspase-3.

7/3,AB/20 (Item 20 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10761665 20304737 PMID: 10844408
Recombinant thrombomodulin inhibits thrombin-induced vascular endothelial growth factor production in neuronal cells.
Sarker K P; Yamahata H; Nakata M; Arisato T; Nakajima T; Kitajima I; Maruyama I
Department of Laboratory and Molecular Medicine, Faculty of Medicine, Kagoshima University, Kagoshima, Japan. sar@khosp2.kufm.kagoshima-u.ac.jp Haemostasis (SWITZERLAND) Nov-Dec 1999, 29 (6) p343-52, ISSN 0301-0147 Journal Code: 0371574
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Thrombin is a serine protease which is generated from its precursor prothrombin by the activation of the blood coagulation cascade. Thrombin converts fibrinogen to fibrin, activates platelets and several coagulation factors, and plays a central role in thrombosis and hemostasis by regulating platelet aggregation and blood coagulation. Here, we show that thrombn enhanced vascular endothelial growth factor (*VEGF*) production in a dose- and time-dependent manner in the supernatant of cultured PC-12 cells, as determined by enzyme-linked immunosorbent assay (ELISA). Thrombin receptor agonist peptide (SFLLRNPNDKYEPF, TRAP) exerted an effect similar to thrombin on *VEGF* production. Thrombin-induced *VEGF* production was significantly attenuated by recombinant human thrombomodulin (rTM) and its minimal functional domain E456. Furthermore, the antioxidant N-acetyl-L-*cysteine* (NAC) markedly inhibited thrombin-induced *VEGF* production. Thus, rTM and NAC apparently inhibited the effect of thrombin on *VEGF* production in neuronal cells. Copyright 2000 S. Karger AG, Basel

7/3,AB/21 (Item 21 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10734748 20281192 PMID: 10823686
Effects of sulfhydryl compounds on interleukin-1-induced vascular endothelial growth factor production in human synovial stromal cells. Tsuji F; Matsuoka H; Aono H; Takai M; Horiuchi M; Nishimura K; Mita S Discovery Research Division, Santen Pharmaceutical Co., Ltd., Osaka, Japan. ftsuji@msn.com
Biological & pharmaceutical bulletin (JAPAN) May 2000, 23 (5) p663-5 , ISSN 0918-6158 Journal Code: 9311984
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
We investigated the effects of various sulfhydryl compounds on interleukin-1 (IL-1)-induced vascular endothelial growth factor (*VEGF*) production in human synovial stromal cells (HSSC). HSSC stimulated by IL-1beta (100 ng/ml) produced *VEGF* and interleukin-6 (IL-6) in vitro. Monosulfhydryl compounds, N-acetylcysteine, D-penicillamine, tiopronin and the bucillamine-like disulfhydryl compound, compound A scarcely affected *VEGF* or IL-6 production at concentrations of 10(-5) and 10(-4) M. However, the disulfhydryl compound, bucillamine inhibited *VEGF* production but not IL-6 production at concentrations of 10(-5) and 10(-4) M. These results suggest that bucillamine may be a selective inhibitor of IL-1-induced *VEGF* production in HSSC, and that inhibition of *VEGF* production may require not only SH groups but also a specific chemical structure.

7/3,AB/22 (Item 22 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10347024 99340059 PMID: 10409677
Differential binding of vascular endothelial growth factor B splice and proteolytic isoforms to neuropilin-1.
Makinen T; Olofsson B; Karpanen T; Hellman U; Soker S; Klagsbrun M; Eriksson U; Alitalo K
Molecular/Cancer Biology Laboratory, Haartman Institute, University of Helsinki, FIN-00014 Helsinki, Finland.
Journal of biological chemistry (UNITED STATES) Jul 23 1999, 274 (30) p21217-22, ISSN 0021-9258 Journal Code: 2985121R
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM

Record type: Completed
Vascular endothelial growth factor B (*VEGF* -B) is expressed in various tissues, especially strongly in the heart, and binds selectively to one of the *VEGF* receptors, VEGFR-1. The two splice isoforms, *VEGF*-B(167) and *VEGF*-B(186), have identical NH(2)-terminal *cystine* knot growth factor domains but differ in their COOH-terminal domains which give these forms their distinct biochemical properties. In this study, we show that both splice isoforms of *VEGF*-B bind specifically to Neuropilin-1 (NRP1), a receptor for collapsins/semaphorins and for the *VEGF*(165) isoform. The NRP1 binding of *VEGF*-B could be competed by an excess of *VEGF*(165). The binding of *VEGF* -B(167) was mediated by the heparin binding domain, whereas the binding of *VEGF* -B(186) to NRP1 was regulated by exposure of a short COOH-terminal proline-rich peptide upon its proteolytic processing. In immunohistochemistry, NRP1 distribution was found to be overlapping or adjacent to known sites of *VEGF*-B expression in several tissues, in particular in the developing heart, suggesting the involvement of *VEGF*-B in NRP1-mediated signaling.

7/3,AB/23 (Item 23 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10157463 99149642 PMID: 10027396
Vascular endothelial growth factor (*VEGF*)-mediated angiogenesis is associated with enhanced endothelial cell survival and induction of Bcl-2 expression.
Nor J E; Christensen J; Mooney D J; Polverini P J
Department of Oral Medicine, Pathology, and Oncology, University of Michigan School of Dentistry, Ann Arbor 48109-1078, USA.
American journal of pathology (UNITED STATES) Feb 1999, 154 (2) p375-84, ISSN 0002-9440 Journal Code: 0370502
Contract/Grant No.: CA64416; CA; NCI; HL39926; HL; NHLBI Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Vascular endothelial growth factor (*VEGF*) is an endothelial cell mitogen and permeability factor that is potently angiogenic in vivo. We report here studies that suggest that *VEGF* potentiates angiogenesis in vivo and prolongs the survival of human dermal microvascular endothelial cells (HDMECs) in vitro by inducing expression of the anti-apoptotic protein Bcl-2. Growth-factor-enriched and serum-deficient cultures of HDMECs grown on collagen type I gels with *VEGF* exhibited a 4-fold and a 1.6-fold reduction, respectively, in the proportion of apoptotic cells. Enhanced HDMEC survival was associated with a dose-dependent increase in Bcl-2 expression and a decrease in the expression of the processed forms of the *cysteine* protease caspase-3. Cultures of HDMECs transduced with and overexpressing Bcl-2 and deprived of growth factors showed enhanced protection from apoptosis and exhibited a twofold increase in cell number and a fourfold increase in the number of capillary-like sprouts. HDMECs overexpressing Bcl-2 when incorporated into polylactic acid sponges and implanted into SCID mice exhibited a sustained fivefold increase in the number of microvessels and a fourfold decrease in the number of apoptotic cells when examined 7 and 14 days later. These results suggest that the angiogenic activity attributed to *VEGF* may be due in part to its ability to enhance endothelial cell survival by inducing expression of Bcl-2.

7/3,AB/24 (Item 24 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10139848 99107753 PMID: 9889193
A novel vascular endothelial growth factor encoded by Orf virus, *VEGF* -E, mediates angiogenesis via signalling through VEGFR-2 (KDR) but not VEGFR-1 (Flt-1) receptor tyrosine kinases.
Meyer M; Clauss M; Lepple-Wienhues A; Waltenberger J; Augustin H G; Ziche M; Lanz C; Buttner M; Rziha H J; Dehio C
Department of Infection Biology, Max Planck Institute for Biology, Spemannstrasse 34, D-72076 Tubingen, Germany.
EMBO journal (ENGLAND) Jan 15 1999, 18 (2) p363-74, ISSN 0261-4189 Journal Code: 8208664
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The different members of the vascular endothelial growth factor (*VEGF*) family act as key regulators of endothelial cell function controlling vasculogenesis, angiogenesis, vascular permeability and endothelial cell survival. In this study, we have functionally characterized a novel member of the *VEGF* family, designated *VEGF*-E. *VEGF* -E sequences are encoded by the parapoxvirus Orf virus (OV). They carry the characteristic *cysteine* knot motif present in all mammalian VEGFs, while forming a microheterogenic group distinct from previously described members of this family. *VEGF* -E was expressed as the native protein in mammalian cells or as a recombinant protein in Escherichia coli and was shown to act as a heat-stable, secreted dimer. *VEGF*-E and *VEGF* -A were found to possess similar bioactivities, i.e. both factors stimulate the release of tissue factor (TF), the proliferation, chemotaxis and sprouting of cultured vascular endothelial cells in vitro and angiogenesis in

vivo. Like *VEGF*-A, *VEGF*-E was found to bind with high affinity to *VEGF* receptor-2 (KDR) resulting in receptor autophosphorylation and a biphasic rise in free intracellular Ca2+ concentration, whilst in contrast to *VEGF*-A, *VEGF*-E did not bind to *VEGF* receptor-1 (Flt-1). *VEGF*-E is thus a potent angiogenic factor selectively binding to *VEGF* receptor-2. These data strongly indicate that activation of *VEGF* receptor-2 alone can efficiently stimulate angiogenesis.

7/3,AB/25 (Item 25 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10057868 99038547 PMID: 9821106
The importance of cell density in the interpretation of growth factor effects on collagenase IV activity release and extracellular matrix production from C6 astrocytoma cells.
Tamaki M; McDonald W; Del Maestro R F
Brain Research Laboratories, London Health Sciences Center, University of Western Ontario, Canada.
Journal of neuro-oncology (NETHERLANDS) Sep 1998, 39 (3) p205-16, ISSN 0167-594X Journal Code: 8309335
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
We have examined the influence of basic fibroblast growth factor (FGF-2) and vascular endothelial growth factor (*VEGF*) on the release of collagenase type IV activity and the production of extracellular matrix (ECM) molecules using C6 astrocytoma cells in monolayer culture. Collagenase type IV activity was significantly increased in a dose dependent manner in the low cell density group by treatment with FGF-2 and *VEGF* but significantly decreased in a dose dependent fashion in the high cell density group. These results were corroborated using Western blot technique with an antibody to gelatinase A. Addition of exogenous laminin and fibronectin to the media decreased collagenase type IV activity in a dose dependent fashion with the minimum concentration of 0.1 microgram/ml. Laminin and fibronectin reached a concentration of 0.1 microgram/ml in only the high cell density group after treatment with the growth factors tested. These findings indicate that C6 astrocytoma cells appear to have two regulatory mechanisms for collagenase type IV activity which are dependent on cell density. In a low cell density, C6 astrocytoma cells respond to the dominant effect of FGF-2 and *VEGF* by increasing the release of collagenase IV activity. In a high cell density collagenase type IV activity is decreased due to it's down regulation by released ECM molecules in response to FGF-2 and *VEGF*. These regulatory mechanisms may be crucial to the understanding of the coordination of tumor-associated angiogenesis by malignant glial cells.

7/3,AB/26 (Item 26 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10042195 99023957 PMID: 9804796
Vascular endothelial growth factor regulates endothelial cell survival through the phosphatidylinositol 3'-kinase/Akt signal transduction pathway. Requirement for Flk-1/KDR activation.
Gerber H P; McMurtrey A; Kowalski J; Yan M; Keyt B A; Dixit V; Ferrara N Department of Cardiovascular Research, Genentech, Inc., South San Francisco, California 94080, USA.
Journal of biological chemistry (UNITED STATES) Nov 13 1998, 273 (46) p30336-43, ISSN 0021-9258 Journal Code: 2985121R
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Vascular endothelial growth factor (*VEGF*) has been found to have various functions on endothelial cells, the most prominent of which is the induction of proliferation and differentiation. In this report we demonstrate that *VEGF* or a mutant, selectively binding to the Flk-1/KDR receptor, displayed high levels of survival activity, whereas Flt-1-specific ligands failed to promote survival of serum-starved primary human endothelial cells. This activity was blocked by the phosphatidylinositol 3'-kinase (PI3-kinase)-specific inhibitors wortmannin and LY294002. Endothelial cells cultured in the presence of *VEGF* and the Flk-1/KDR-selective *VEGF* mutant induced phosphorylation of the serine-threonine kinase Akt in a PI3-kinase-dependent manner. Akt activation was not detected in response to stimulation with placenta growth factor or an Flt-1-selective *VEGF* mutant. Furthermore, a constitutively active Akt was sufficient to promote survival of serum-starved endothelial cells in transient transfection experiments. In contrast, overexpression of a dominant-negative form of Akt blocked the survival effect of *VEGF* . These findings identify the Flk-1/KDR receptor and the PI3-kinase/Akt signal transduction pathway as crucial elements in the processes leading to endothelial cell survival induced by *VEGF* . Inhibition of apoptosis may represent a major aspect of the regulatory activity of *VEGF* on the vascular endothelium.

7/3,AB/27 (Item 27 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09981902 98426218 PMID: 9751730

Vascular endothelial growth factor B (*VEGF*-B) binds to *VEGF* receptor-1 and regulates plasminogen activator activity in endothelial cells.
Olofsson B; Korpelainen E; Pepper M S; Mandriota S J; Aase K; Kumar V; Gunji Y; Jeltsch M M; Shibuya M; Alitalo K; Eriksson U
Ludwig Institute for Cancer Research, Stockholm Branch, Box 240, S-171 77 Stockholm, Sweden.
Proceedings of the National Academy of Sciences of the United States of America (UNITED STATES) Sep 29 1998, 95 (20) p11709-14, ISSN 0027-8424 Journal Code: 7505876
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The vascular endothelial growth factor (*VEGF*) family has recently expanded by the identification and cloning of three additional members, namely *VEGF*-B, *VEGF*-C, and *VEGF* -D. In this study we demonstrate that *VEGF*-B binds selectively to *VEGF* receptor-1/Flt-1. This binding can be blocked by excess *VEGF*, indicating that the interaction sites on the receptor are at least partially overlapping. Mutating the putative *VEGF* receptor-1/Flt-1 binding determinants Asp63, Asp64, and Glu67 to alanine residues in *VEGF*-B reduced the affinity to *VEGF* receptor-1 but did not abolish binding. Mutational analysis of conserved cysteines contributing to *VEGF*-B dimer formation suggest a structural conservation with *VEGF* and platelet-derived growth factor. Proteolytic processing of the 60-kDa *VEGF*-B186 dimer results in a 34-kDa dimer containing the receptor-binding epitopes. The binding of *VEGF*-B to its receptor on endothelial cells leads to increased expression and activity of urokinase type plasminogen activator and plasminogen activator inhibitor 1, suggesting a role for *VEGF* -B in the regulation of extracellular matrix degradation, cell adhesion, and migration.

7/3,AB/28 (Item 28 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09923838 98352100 PMID: 9685413
2'-Fluoropyrimidine RNA-based aptamers to the 165-amino acid form of vascular endothelial growth factor (VEGF165). Inhibition of receptor binding and *VEGF*-induced vascular permeability through interactions requiring the exon 7-encoded domain.
Ruckman J; Green L S; Beeson J; Waugh S; Gillette W L; Henninger D D; Claesson-Welsh L; Janjic N
NeXstar Pharmaceuticals, Inc., Boulder, Colorado 80301, USA. Journal of biological chemistry (UNITED STATES) Aug 7 1998, 273 (32) p20556-67, ISSN 0021-9258 Journal Code: 2985121R
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Vascular endothelial growth factor (*VEGF*) has been implicated in the pathological induction of new blood vessel growth in a variety of proliferative disorders. Using the SELEX process (systematic evolution of ligands by exponential enrichment), we have isolated 2'-F-pyrimidine RNA oligonucleotide ligands (aptamers) to human VEGF165. Representative aptamers from three distinct sequence families were truncated to the minimal sequence capable of high affinity binding to *VEGF* (23-29 nucleotides) and were further modified by replacement of 2'-O-methyl for 2'-OH at all ribopurine positions where the substitution was tolerated. Equilibrium dissociation constants for the interaction of *VEGF* with the truncated, 2'-O-methyl-modified aptamers range between 49 and 130 pM. These aptamers bind equally well to murine VEGF164, do not bind to VEGF121 or the smaller isoform of placenta growth factor (PlGF129), and show reduced, but significant affinity for the VEGF165/PlGF129 heterodimer. *Cysteine* 137 in the exon 7-encoded domain of VEGF165 forms a photo-inducible cross-link to a single uridine residue in each of the three aptamers. The aptamers potently inhibit the binding of *VEGF* to the human *VEGF* receptors, KDR and Flt-1, expressed by transfected porcine aortic endothelial cells. Furthermore, one of the aptamers is able to significantly reduce intradermal *VEGF*-induced vascular permeability in vivo.

7/3,AB/29 (Item 29 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09881480 98311257 PMID: 9648923
Amino acid depletion modulates vascular endothelial growth factor production during the life span of human vascular smooth muscle cells. Earle K A; Pancholi S; Vernon P; Yudkin J S
Department of Medicine, Centre for Diabetes and Cardiovascular Risk, University College London, Whittington Hospital, United Kingdom. k.earle@ucl.ac.uk
Journal of cellular physiology (UNITED STATES) Aug 1998, 176 (2) p359-64, ISSN 0021-9541 Journal Code: 0050222
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The role of nutrient supply in the replicative capacity and secretory phenotype of cultured human diploid

cells is unclear. We examined the relationship between amino acid privation, the secretion of vascular endothelial growth factor (*VEGF*) and growth phenotype of vascular smooth muscle cells (VSMC), and endothelial cells. Cultures of VSMCs, but not endothelial cells, were growth inhibited by exposure to medium that was 75% deficient in leucine, methionine, arginine, and *cysteine* over two passages. Exposed VSMC cultures exhibited an increased vulnerability to apoptosis. The maximal cumulative population doubling of the exposed cells was reduced significantly compared with the control cells (25.7 +/- 2.0 doublings vs. 27.9 +/- 2.1 doublings; P < 0.03). Constitutive *VEGF* production first became evident in the later passages of the exposed and nonexposed cell cultures. However, production of *VEGF* was 17-fold greater in the exposed cultures at the tenth passage (P < 0.001). The replicative capacity and constitutive production of *VEGF* in VSMCs in culture may be programmed by transient privation of amino acids. These observations are relevant to new concepts concerning the pathogenesis of vascular disease.

7/3,AB/30 (Item 30 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09710299 98126042 PMID: 9466566
Activation of coagulation and angiogenesis in cancer: immunohistochemical localization in situ of clotting proteins and vascular endothelial growth factor in human cancer.
Shoji M; Hancock W W; Abe K; Micko C; Casper K A; Baine R M; Wilcox J N; Danave I; Dillehay D L; Matthews E; Contrino J; Morrissey J H; Gordon S; Edgington T S; Kudryk B; Kreutzer D L; Rickles F R
Hematologic Disease Branch, DASTLR/NCID, Centers for Disease Control and Prevention, Atlanta, Georgia 30333, USA.
American journal of pathology (UNITED STATES) Feb 1998, 152 (2) p399-411, ISSN 0002-9440 Journal Code: 0370502
Contract/Grant No.: CA22202; CA; NCI; DK44827; DK; NIDDK; EYE04131; EY; NEI; +
Document type: Journal Article; Review; Review, Tutorial
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Thrombin-catalyzed, cross-linked fibrin (XLF) formation is a characteristic histopathological finding in many human and experimental tumors and is thought to be of importance in the local host defense response. Although the pathogenesis of tumor-associated fibrin deposition is not entirely clear, several tumor procoagulants have been described as likely primary stimuli for the generation of thrombin (and XLF) in the tumor microenvironment (TME). In a previous study of a variety of human tumors we have shown that tissue factor (TF) is the major procoagulant. However, the relative contribution to fibrin deposition in the TME of tumor cell TF and host cell TF (eg, macrophage-derived) was not established. In addition, recent evidence has implicated TF in the regulation of the synthesis of the pro-angiogenic factor vascular endothelial growth factor (*VEGF*) by tumor cells. In the current study we used in situ techniques to determine the cellular localization of XLF, TF, *VEGF*, and an alternative tumor procoagulant, so-called cancer procoagulant (CP), a *cysteine* protease that activates clotting factor X. In lung cancer we have found XLF localized predominantly to the surface of tumor-associated macrophages, as well as to some endothelial cells and perivascular fibroblasts in the stromal area of the tumors co-distributed with TF at the interface of the tumor and host cells. Cancer pro-coagulant was localized to tumor cells in several cases but not in conjunction with the deposition of XLF. TF and *VEGF* were co-localized in both lung cancer and breast cancer cells by in situ hybridization and immunohistochemical staining. Furthermore, a strong relationship was found between the synthesis of TF and *VEGF* levels in human breast cancer cell lines (r2 = 0.84; P < 0.0001). Taken together, these data are consistent with a highly complex interaction between tumor cells, macrophages, and endothelial cells in the TME leading to fibrin formation and tumor angiogenesis.

7/3,AB/31 (Item 31 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09631932 98058949 PMID: 9395496
Inhibition of vascular endothelial growth factor (*VEGF*)-induced endothelial cell proliferation by a peptide corresponding to the exon 7-encoded domain of VEGF165.
Soker S; Gollamudi-Payne S; Fidder H; Charmahelli H; Klagsbrun M Department of Surgery, Children's Hospital, Boston, Massachusetts 02115, USA.
Journal of biological chemistry (UNITED STATES) Dec 12 1997, 272 (50) p31582-8, ISSN 0021-9258 Journal Code: 2985121R
Contract/Grant No.: CA37392; CA; NCI; GM47397; GM; NIGMS Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Vascular endothelial growth factor (*VEGF*) is a potent mitogen for endothelial cells (EC) in vitro and a major regulator of angiogenesis in vivo. VEGF121 and VEGF165 are the most abundant of the five known *VEGF* isoforms. The structural difference between these two is the presence in VEGF165 of 44 amino acids

encoded by exon 7 lacking in VEGF121. It was previously shown that VEGF165 and VEGF121 both bind to KDR/Flk-1 and Flt-1 but that VEGF165 binds in addition to a novel receptor (Soker, S., Fidder, H., Neufeld, G., and Klagsbrun, M. (1996) J. Biol. Chem. 271, 5761-5767). The binding of VEGF165 to this VEGF165-specific receptor (VEGF165R) is mediated by the exon 7-encoded domain. To investigate the biological role of this domain further, a glutathione S-transferase fusion protein corresponding to the VEGF165 exon 7-encoded domain was prepared. The fusion protein inhibited binding of 125I-VEGF165 to VEGF165R on human umbilical vein-derived EC (HUVEC) and MDA-MB-231 tumor cells. The fusion protein also inhibited significantly 125I-VEGF165 binding to KDR/Flk-1 on HUVEC but not on porcine EC which express KDR/Flk-1 alone. VEGF165 had a 2-fold higher mitogenic activity for HUVEC than did VEGF121. The exon 7 fusion protein inhibited VEGF165-induced HUVEC proliferation by 60% to about the level stimulated by VEGF121. Unexpectedly, the fusion protein also inhibited HUVEC proliferation in response to VEGF121. Deletion analysis revealed that a core inhibitory domain exists within the C-terminal 23-amino acid portion of the exon 7-encoded domain and that a *cysteine* residue at position 22 in exon 7 is critical for inhibition. It was concluded that the exon 7-encoded domain of VEGF165 enhances its mitogenic activity for HUVEC by interacting with VEGF165R and modulating KDR/Flk-1-mediated mitogenicity indirectly and that exon 7-derived peptides may be useful *VEGF* antagonists in angiogenesis-associated diseases.

7/3,AB/32 (Item 32 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09627311 98035455 PMID: 9351807
The crystal structure of vascular endothelial growth factor (*VEGF*) refined to 1.93 A resolution: multiple copy flexibility and receptor binding.
Muller Y A; Christinger H W; Keyt B A; de Vos A M
Department of Protein Engineering, Genentech, Inc., South San Francisco, CA 94080, USA.
Structure (London, England) (ENGLAND) Oct 15 1997, 5 (10) p1325-38, ISSN 0969-2126 Journal Code: 9418985
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
BACKGROUND: Vascular endothelial growth factor (*VEGF*) is an endothelial cell-specific angiogenic and vasculogenic mitogen. *VEGF* also plays a role in pathogenic vascularization which is associated with a number of clinical disorders, including cancer and rheumatoid arthritis. The development of *VEGF* antagonists, which prevent the interaction of *VEGF* with its receptor, may be important for the treatment of such disorders. *VEGF* is a homodimeric member of the *cystine* knot growth factor superfamily, showing greatest similarity to platelet-derived growth factor (PDGF). *VEGF* binds to two different tyrosine kinase receptors, kinase domain receptor (KDR) and Fms-like tyrosine kinase 1 (Flt-1), and a number of *VEGF* homologs are known with distinct patterns of specificity for these same receptors. The structure of *VEGF* will help define the location of the receptor-binding site, and shed light on the differences in specificity and cross-reactivity among the *VEGF* homologs. RESULTS: We have determined the crystal structure of the receptor-binding domain of *VEGF* at 1.93 A resolution in a triclinic space group containing eight monomers in the asymmetric unit. Superposition of the eight copies of *VEGF* shows that the beta-sheet core regions of the monomers are very similar, with slightly greater differences in most loop regions. For one loop, the different copies represent different snapshots of a concerted motion. Mutagenesis mapping shows that this loop is part of the receptor-binding site of *VEGF*. CONCLUSIONS: A comparison of the eight independent copies of *VEGF* in the asymmetric unit indicates the conformational space sampled by the protein in solution; the root mean square differences observed are similar to those seen in ensembles of the highest precision NMR structures. Mapping the receptor-binding determinants on a multiple sequence alignment of *VEGF* homologs, suggests the differences in specificity towards KDR and Flt-1 may derive from both sequence variation and changes in the flexibility of binding loops. The structure can also be used to predict possible receptor-binding determinants for related *cystine* knot growth factors, such as PDGF.

7/3,AB/33 (Item 33 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09571991 97460110 PMID: 9312130
Genomic organization of human and mouse genes for vascular endothelial growth factor C.
Chilov D; Kukk E; Taira S; Jeltsch M; Kaukonen J; Palotie A; Joukov V; Alitalo K
Molecular/Cancer Biology Laboratory, Haartman Institute, PL21 (Haartmaninkatu 3), University of Helsinki, Helsinki 00014, Finland. Journal of biological chemistry (UNITED STATES) Oct 3 1997, 272 (40) p25176-83, ISSN 0021-9258 Journal Code: 2985121R
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
We report here the cloning and characterization of human and mouse genes for vascular endothelial growth

factor C (*VEGF*-C), a newly isolated member of the vascular endothelial growth factor/platelet-derived growth factor (*VEGF*/PDGF) family. Both *VEGF*-C genes comprise over 40 kilobase pairs of genomic DNA and consist of seven exons, all containing coding sequences. The *VEGF* homology domain of *VEGF*-C is encoded by exons 3 and 4. Exons 5 and 7 encode *cysteine*-rich motifs of the type C6C10CRC, and exon 6 encodes additional C10CXCXC motifs typical of a silk protein. A putative alternatively spliced rare RNA form lacking exon 4 was identified in human fibrosarcoma cells, and a major transcription start site was located in the human *VEGF*-C gene 523 base pairs upstream of the translation initiation codon. The upstream promoter sequences contain conserved putative binding sites for Sp-1, AP-2, and NF-kappaB transcription factors but no TATA box, and they show promoter activity when transfected into cells. The *VEGF*-C gene structure is thus assembled from exons encoding propeptides and distinct *cysteine*-rich domains in addition to the *VEGF* homology domain, and it shows both similarities and distinct differences in comparison with other members of the *VEGF* /PDGF gene family.

7/3,AB/34 (Item 34 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09499292 97399482 PMID: 9255609
VEGF mRNA is stabilized by ras and tyrosine kinase oncogenes, as well as by UV radiation--evidence for divergent stabilization pathways. White F C; Benehacene A; Scheele J S; Kamps M
Department of Pathology, UCSD School of Medicine, La Jolla 92093-0612, USA.
Growth factors (Chur, Switzerland) (SWITZERLAND) 1997, 14 (2-3) p199-212, ISSN 0897-7194 Journal Code: 9000468
Contract/Grant No.: 2PO1 CA50528; CA; NCI; HL54451-02; HL; NHLBI Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Vascular Endothelial Growth Factor (*VEGF*) is a pivotal endothelial cell mitogen that mediates both normal and pathological angiogenesis. Although expressed at very low levels in cells not undergoing vascularization, *VEGF* mRNA is transiently upregulated and stabilized by a variety of extracellular stimuli, and is persistently upregulated and stabilized in many human tumor cell lines (White et al., 1995). Here we demonstrate that oncogenic activation of tyrosine protein kinases and Ras proteins induce a 6- to 16-fold increase in the abundance of *VEGF* mRNA and a 3- to 5-fold increase in the stability of *VEGF* mRNA, suggesting that persistent activation of signaling pathways induced by these oncoproteins accounts for overexpression of *VEGF* in a significant fraction of human tumors. In addition to these oncoproteins, ultraviolet (UV) radiation upregulated and stabilized *VEGF* mRNA 15- and 5-fold, respectively. While the tyrosine kinase inhibitor, genistein, blocked *VEGF* upregulation by activated tyrosine protein kinases, and the Ras inhibitor, N-Acetyl-S-trans-farnesyl-L-*cysteine* (AFC), eliminated *VEGF* expression in cells transformed by v-Ras, neither agent blocked upregulation by hypoxia or UV radiation. These data argue that multiple divergent pathways upregulate and stabilize *VEGF* mRNA.

7/3,AB/35 (Item 35 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09479672 97377029 PMID: 9233800
Proteolytic processing regulates receptor specificity and activity of *VEGF*-C.
Joukov V; Sorsa T; Kumar V; Jeltsch M; Claesson-Welsh L; Cao Y; Saksela O ; Kalkkinen N; Alitalo K
Molecular/Cancer Biology Laboratory, University of Helsinki, Finland. EMBO journal (ENGLAND) Jul 1 1997, 16 (13) p3898-911, ISSN 0261-4189 Journal Code: 8208664
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The recently identified vascular endothelial growth factor C (*VEGF* -C) belongs to the platelet-derived growth factor (PDGF)/*VEGF* family of growth factors and is a ligand for the endothelial-specific receptor tyrosine kinases VEGFR-3 and VEGFR-2. The *VEGF* homology domain spans only about one-third of the *cysteine*-rich *VEGF*-C precursor. Here we have analysed the role of post-translational processing in *VEGF*-C secretion and function, as well as the structure of the mature *VEGF*-C. The stepwise proteolytic processing of *VEGF*-C generated several *VEGF* -C forms with increased activity towards VEGFR-3, but only the fully processed *VEGF*-C could activate VEGFR-2. Recombinant 'mature' *VEGF* -C made in yeast bound VEGFR-3 (K[D] = 135 pM) and VEGFR-2 (K[D] = 410 pM) and activated these receptors. Like *VEGF*, mature *VEGF* -C increased vascular permeability, as well as the migration and proliferation of endothelial cells. Unlike other members of the PDGF/*VEGF* family, mature *VEGF*-C formed mostly non-covalent homodimers. These data implicate proteolytic processing as a regulator of *VEGF*-C activity, and reveal novel structure-function relationships in the PDGF/*VEGF* family.

7/3,AB/36 (Item 36 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09476609 97388482 PMID: 9247316
Characterization of murine Flt4 ligand/*VEGF*-C.
Fitz L J; Morris J C; Towler P; Long A; Burgess P; Greco R; Wang J; Gassaway R; Nickbarg E; Kovacic S; Ciarletta A; Giannotti J; Finnerty H; Zollner R; Beier D R; Leak L V; Turner K J; Wood C R
Genetics Institute, Inc., Cambridge, Massachusetts 02140, USA. Oncogene (ENGLAND) Jul 31 1997, 15 (5) p613-8, ISSN 0950-9232 Journal Code: 8711562
Contract/Grant No.: HD 29028; HD; NICHD; RO1 HG00951; HG; NHGRI Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Flt4 is a receptor protein tyrosine kinase that is expressed in the adult lymphatic endothelium and high endothelial venules. We have used a BIAcore assay to identify rodent and human cell conditioned media containing the ligand of Flt4 (Flt4-L). Receptor-based affinity chromatography was used to purify this growth factor, followed by amino acid sequencing and molecular cloning of the murine cDNA, the orthologue of human vascular endothelial growth factor-C and vascular endothelial growth factor related protein. The murine flt4-L gene was localized to chromosome 8 and demonstrated to be widely expressed. Flt4-L was found to have a hydrophobic signal sequence and a pro-peptide-like sequence that is removed to generate the mature N-terminus. In addition, the C-terminal region of Flt4-L has four repeats of a *cysteine* -rich motif that is presumably also proteolytically processed to generate the 21000 Mr polypeptide subunit of the Flt4-L homodimer. Recombinant Flt4-L activated Flt4 as judged by induction of tyrosyl phosphorylation, and induced mitogenesis in vitro of lymphatic endothelial cells.

7/3,AB/37 (Item 37 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09456738 97349118 PMID: 9205122
Molecular cloning of a novel vascular endothelial growth factor, *VEGF*-D.
Yamada Y; Nezu J; Shimane M; Hirata Y
Gene Search Program, Chugai Research Institute for Molecular Medicine, Niihari, Ibaraki, Japan. yamaday@tk.chugai-pharm.co.jp
Genomics (UNITED STATES) Jun 15 1997, 42 (3) p483-8, ISSN 0888-7543 Journal Code: 8800135
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
We have identified and characterized a novel vascular endothelial growth factor (*VEGF*), *VEGF* -D, which is structurally related to vascular endothelial growth factor C. A full-length cDNA for human *VEGF* -D was cloned following the identification of an EST obtained through a TFASTA search of public EST databases. The murine *VEGF*-D was subsequently isolated from a mouse lung cDNA library. The human *VEGF* -D gene was mapped to human chromosome Xp22.31. Both human and mouse *VEGF*-D are strongly expressed in lung and encode the eight *cysteine* residues that are highly conserved among the members of this family. The high level of conservation between mouse and human *VEGF*-D may emphasize the biological importance of this gene. Recently the murine gene, FIGF, which is identical to mouse *VEGF*-D, was reported.

7/3,AB/38 (Item 38 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09448614 97352774 PMID: 9207067
Vascular endothelial growth factor: crystal structure and functional mapping of the kinase domain receptor binding site.
Muller Y A; Li B; Christinger H W; Wells J A; Cunningham B C; de Vos A M Genentech, Inc., Department of Protein Engineering, 460 Point San Bruno Boulevard, South San Francisco, CA 94080, USA.
Proceedings of the National Academy of Sciences of the United States of America (UNITED STATES) Jul 8 1997, 94 (14) p7192-7, ISSN 0027-8424 Journal Code: 7505876
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Vascular endothelial growth factor (*VEGF*) is a homodimeric member of the *cystine* knot family of growth factors, with limited sequence homology to platelet-derived growth factor (PDGF) and transforming growth factor beta2 (TGF-beta). We have determined its crystal structure at a resolution of 2.5 A, and identified its kinase domain receptor (KDR) binding site using mutational analysis. Overall, the *VEGF* monomer resembles that of PDGF, but its N-terminal segment is helical rather than extended. The dimerization mode of *VEGF* is similar to that of PDGF and very different from that of TGF-beta. Mutational analysis of *VEGF* reveals that symmetrical binding sites for KDR are located at each pole of the *VEGF* homodimer. Each site contains two functional "hot spots" composed of binding determinants presented across the subunit interface. The two most important determinants are located within the largest hot spot on a short, three-stranded sheet that is conserved in PDGF and

TGF-beta. Functional analysis of the binding epitopes for two receptor-blocking antibodies reveal different binding determinants near each of the KDR binding hot spots.

7/3,AB/39 (Item 39 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09215878 97111903 PMID: 8953654
Crystallization of the receptor binding domain of vascular endothelial growth factor.
Christinger H W; Muller Y A; Berleau L T; Keyt B A; Cunningham B C; Ferrara N; de Vos A M
Department of Protein Engineering, Genentech, Inc., South San Francisco, California 94080, USA.
Proteins (UNITED STATES) Nov 1996, 26 (3) p353-7, ISSN 0887-3585 Journal Code: 8700181
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Vascular endothelial growth factor (*VEGF*) is a potent angiogenic factor with a unique specificity for vascular endothelial cells. In addition to its role in vasculogenesis and embryonic angiogenesis, *VEGF* is implicated in pathologic neovascularization associated with tumors and diabetic retinopathy. Four different constructs of a short variant of *VEGF* sufficient for receptor binding were overexpressed in Escherichia coli, refolded, purified, and crystallized in five different space groups. In order to facilitate the production of heavy atom derivatives, single *cysteine* mutants were designed based on the crystal structure of platelet-derived growth factor. A construct consisting of residues 8 to 109 was crystallized in space group P2(1), with cell parameters a = 55.6 A, b = 60.4 A, c = 77.7 A, beta = 90.0 degrees, and four monomers in the asymmetric unit. Native and derivative data were collected for two of the *cysteine* mutants as well as for wild-type *VEGF*.

7/3,AB/40 (Item 40 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09179088 97059399 PMID: 8903720
The placenta growth factor gene of the mouse.
DiPalma T; Tucci M; Russo G; Maglione D; Lago C T; Romano A; Saccone S; Della Valle G; De Gregorio L; Dragani T A; Viglietto G; Persico M G International Institute of Genetics and Biophysics, Naples, Italy.
Mammalian genome : official journal of the International Mammalian Genome Society (UNITED STATES) Jan 1996, 7 (1) p6-12, ISSN 0938-8990 Journal Code: 9100916
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Placenta growth factor (PlGF) and vascular endothelial growth factor (*VEGF*) are angiogenic factors containing the 8-*cysteine* motif of platelet-derived growth factor (PDGF). Both PlGF and *VEGF* are mitogens for endothelial cells in vitro and promote neoangiogenesis in vivo. In addition, PlGF strongly potentiates the proliferative and the permeabilization effects exerted by *VEGF* on the vascular endothelium. We have now isolated the cDNA coding for mouse Plgf by screening a mouse heart cDNA library with the human PlGF sequence as probe. The human PlGF protein has two forms, PlGF-1 and PlGF-2, that arise from alternative splicing of a single gene mapping on Chromosome (Chr) 14; the isolated mouse Plgf cDNA encodes the longer of these two forms (PlGF-2). We show that the mouse Plgf-2 mRNA is the only transcript present in the normal tissues analyzed. Mouse Plgf-2 is a 158-amino-acid-long protein that shows 78% similarity (65% identity) to the human PlGF-2. Computer analysis reveals a putative signal peptide and three probable N-glycosylation sites, two of which are also conserved in human PlGF. The mouse Plgf gene was isolated and characterized; the gene is encoded by 7 exons spanning a 13-kb DNA interval. Finally, we have mapped the mouse Plgf gene to Chr 12, one cM from D12Mit5, and the human PlGF gene to 14q24, using both FISH and genetic crosses.

7/3,AB/41 (Item 41 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09164251 97077124 PMID: 8919691
Cloning and characterization of a novel human gene related to vascular endothelial growth factor.
Grimmond S; Lagercrantz J; Drinkwater C; Silins G; Townson S; Pollock P; Gotley D; Carson E; Rakar S; Nordenskjold M; Ward L; Hayward N; Weber G Queensland Cancer Fund Research Unit Joint Experimental Oncology Program, Queensland Institute of Medical Research, Herston, Australia. seanG@qimr.edu.au
Genome research (UNITED STATES) Feb 1996, 6 (2) p124-31, ISSN 1088-9051 Journal Code: 9518021
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
This paper describes the cloning and characterization of a new member of the vascular endothelial growth factor (*VEGF*) gene family, which we have designated VRF for *VEGF*-related-factor. Sequencing of cDNAs

from a human fetal brain library and RT-PCR products from normal and tumor tissue cDNA pools indicate two alternatively spliced messages with open reading frames of 621 and 564 bp, respectively. The predicted proteins differ at their carboxyl ends resulting from a shift in the open reading frame. Both isoforms show strong homology to *VEGF* at their amino termini, but only the shorter isoform maintains homology to *VEGF* at its carboxyl terminus and conserves all 16 *cysteine* residues of VEGF165. Similarity comparisons of this isoform revealed overall protein identity of 48% and conservative substitution of 69% with VEGF189. VRF is predicted to contain a signal peptide, suggesting that it may be a secreted factor. The VRF gene maps to the D11S750 locus at chromosome band 11q13, and the protein coding region, spanning approximately 5 kb, is comprised of 8 exons that range in size from 36 to 431 bp. Exons 6 and 7 are contiguous and the two isoforms of VRF arise through alternate splicing of exon 6. VRF appears to be ubiquitously expressed as two transcripts of 2.0 and 5.5 kb; the level of expression is similar among normal and malignant tissues.

7/3,AB/42 (Item 42 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08959059 96312526 PMID: 8700872
Vascular endothelial growth factor-related protein: a ligand and specific activator of the tyrosine kinase receptor Flt4.
Lee J; Gray A; Yuan J; Luoh S M; Avraham H; Wood W I
Department of Molecular Biology, Genetech, Inc., South San Francisco, CA 94080, USA.
Proceedings of the National Academy of Sciences of the United States of America (UNITED STATES) Mar 5 1996, 93 (5) p1988-92, ISSN 0027-8424 Journal Code: 7505876
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The tyrosine kinases Flt4, Flt1, and Flk1 (or KDR) constitute a family of endothelial cell-specific receptors with seven immunoglobulin-like domains and a split kinase domain. Flt1 and Flk1 have been shown to play key roles in vascular development; these two receptors bind and are activated by vascular endothelial growth factor (*VEGF*). No ligand has been identified for Flt4, whose expression becomes restricted during development to the lymphatic endothelium. We have identified cDNA clones from a human glioma cell line that encode a secreted protein with 32% amino acid identity to *VEGF*. This protein, designated *VEGF*-related protein (VRP), specifically binds to the extracellular domain of Flt4, stimulates the tyrosine phosphorylation of Flt4 expressed in mammalian cells, and promotes the mitogenesis of human lung endothelial cells. VRP fails to bind appreciably to the extracellular domain of Flt1 or Flk1. The protein contains a C-terminal, *cysteine*-rich region of about 180 amino acids that is not found in *VEGF*. A 2.4-kb VRP mRNA is found in several human tissues including adult heart, placenta, ovary, and small intestine and in fetal lung and kidney.

7/3,AB/43 (Item 43 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08827539 96178224 PMID: 8617204
A novel vascular endothelial growth factor, *VEGF*-C, is a ligand for the Flt4 (VEGFR-3) and KDR (VEGFR-2) receptor tyrosine kinases. Joukov V; Pajusola K; Kaipainen A; Chilov D; Lahtinen I; Kukk E; Saksela O; Kalkkinen N; Alitalo K
Department of Virology, Haartman Institute, University of Helinski, Finland.
EMBO journal (ENGLAND) Jan 15 1996, 15 (2) p290-98, ISSN 0261-4189 Journal Code: 8208664
Erratum in EMBO J 1996 Apr 1;15(7) 1751
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Angiogenesis, the sprouting of new blood vessels from pre-existing ones, and the permeability of blood vessels are regulated by vascular endothelial growth factor (*VEGF*) via its two known receptors Flt1 (VEGFR-1) and KDR/Flk-1 (VEGFR-2). The Flt4 receptor tyrosine kinase is related to the *VEGF* receptors, but does not bind *VEGF* and its expression becomes restricted mainly to lymphatic endothelia during development. In this study, we have purified the Flt4 ligand, *VEGF*-C, and cloned its cDNA from human prostatic carcinoma cells. While *VEGF*-C is homologous to other members of the *VEGF*/platelet derived growth factor (PDGF) family, its C-terminal half contains extra *cysteine*-rich motifs characteristic of a protein component of silk produced by the larval salivary glands of the midge, Chironomus tentans. *VEGF* -C is proteolytically processed, binds Flt4, which we rename as VEGFR-3 and induces tyrosine autophosphorylation of VEGFR-3 and VEGFR-2. In addition, *VEGF*-C stimulated the migration of bovine capillary endothelial cells in collagen gel. *VEGF*-C is thus a novel regulator of endothelia, and its effects may extend beyond the lymphatic system, where Flt4 is expressed.

7/3,AB/44 (Item 44 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08365470 95110840 PMID: 7811723

Structural requirements for dimerization, glycosylation, secretion, and biological function of VPF/*VEGF*.

Claffey K P; Senger D R; Spiegelman B M

Department of Pathology, Beth Israel Hospital, Boston, MA 02215. Biochimica et biophysica acta (NETHERLANDS) Jan 5 1995, 1246 (1) p1-9, ISSN 0006-3002 Journal Code: 0217513

Contract/Grant No.: CA 43967; CA; NCI; DK 08278; DK; NIDDK; DK 42420; DK; NIDDK

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Vascular permeability factor (VPF) also known as vascular endothelial growth factor (*VEGF*), is a dimeric protein that affects endothelial cell (EC) and vascular functions including enhancement of microvascular permeability and stimulation of EC growth. To investigate the structural features of VPF/*VEGF* necessary for efficient dimerization, secretion, and biological activities, we employed site-directed mutagenesis with a Cos-1 cell expression system. Several *cysteine* residues essential for VPF dimerization were identified by mutation analysis of the Cys-25, Cys-56, and Cys-67 residues. Mutant VPF isoforms lacking either of these cysteines were secreted as monomers and were completely inactive in both vascular permeability and endothelial cell mitotic assays. VPF Cys-145 mutant protein was efficiently secreted as a glycosylated, dimeric polypeptide, but had a reduction in biological activities. The site of N-linked glycosylation was directly identified as Asn-74, which, when mutated produced an inefficiently secreted dimeric protein without post-translational glycosylation, yet maintained full vascular permeability activity. Finally, we found that one VPF mutant isoform Cys-101 was not secreted and this mutant functioned as a dominant-negative suppressor of wild-type VPF secretion as demonstrated by co-expression assays in Cos-1 cells.

7/3,AB/45 (Item 45 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07574249 93100326 PMID: 1464614

Dual regulation of vascular endothelial growth factor bioavailability by genetic and proteolytic mechanisms.

Houck K A; Leung D W; Rowland A M; Winer J; Ferrara N

Department of Molecular Biology, Genetech, Inc., South San Francisco, California 94080.

Journal of biological chemistry (UNITED STATES) Dec 25 1992, 267 (36) p26031-7, ISSN 0021-9258 Journal Code: 2985121R

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

The vascular endothelial growth factor (*VEGF*) family encompasses four polypeptides that result from alternative splicing of mRNA. We have previously demonstrated differences in the secretion pattern of these polypeptides. Stable cell lines expressing VEGFs were established in human embryonic kidney CEN4 cells. VEGF121, the shortest form, was secreted and freely soluble in tissue culture medium. VEGF189 was secreted, but was almost entirely bound to the cell surface or extracellular matrix. VEGF165 displayed an intermediary behavior. Suramin induced the release of VEGF189, permitting its characterization as a more basic protein with higher affinity for heparin than VEGF165 or VEGF121, but with similar endothelial cell mitogenic activity. Heparin, heparan sulfate, and heparinase all induced the release of VEGF165 and VEGF189, suggesting heparin-containing proteoglycans as candidate *VEGF*-binding sites. Finally, VEGF165 and VEGF189 were released from their bound states by treatment with plasmin. The released 34-kDa dimeric species are active as endothelial cell mitogens and as vascular permeability agents. We conclude that the bioavailability of *VEGF* may be regulated at the genetic level by alternative splicing that determines whether *VEGF* will be soluble or incorporated into a biological reservoir and also through proteolysis following plasminogen activation.

7/3,AB/46 (Item 46 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

06437560 90121225 PMID: 2610687

Vascular endothelial growth factor: a new member of the platelet-derived growth factor gene family.

Tischer E; Gospodarowicz D; Mitchell R; Silva M; Schilling J; Lau K; Crisp T; Fiddes J C; Abraham J A

California Biotechnology Inc., Mountain View 94043.

Biochemical and biophysical research communications (UNITED STATES) Dec 29 1989, 165 (3) p1198-206, ISSN 0006-291X Journal Code: 0372516 Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Using applications of the polymerase chain reaction (PCR) technique, cDNA clones have been isolated encoding bovine vascular endothelial growth factor (*VEGF*), a mitogen with specificity for vascular endothelial cells. Analysis of the clones indicates that *VEGF* can exist in two forms, probably due to alternative RNA splicing. The amino acid sequences

predicted from the clones also show that *VEGF* shares homologies of about 21% and 24% respectively with the A and B chains of human platelet-derived growth factor (PDGF), and has complete conservation of the eight *cysteine* residues found in both mature PDGF chains. The homology is not reflected in function, however, since the cell types responsive to *VEGF* are distinct from those responsive to homo- and heterodimers of the PDGF chains.

7/3,AB/47 (Item 1 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
(c) 2002 Inst for Sci Info. All rts. reserv.

10523490 Genuine Article#: 536NT Number of References: 35 Title: Increased fibrovascular invasion of subcutaneous polyvinyl alcohol sponges in SPARC-null mice (ABSTRACT AVAILABLE)
Author(s): Bradshaw AD; Reed MJ; Carbon JG; Pinney E; Brekken RA; Sage EH (REPRINT)
Corporate Source: Hope Heart Inst,Dept Vasc Biol,1124 Columbia St,Ste 723/Seattle//WA/98104 (REPRINT); Hope Heart Inst,Dept Vasc Biol,Seattle//WA/98104; Univ Washington,Dept Med,Seattle//WA/; Adv Tissue Sci,La Jolla//CA/
Journal: WOUND REPAIR AND REGENERATION, 2001, V9, N6 (NOV-DEC), P522-530 ISSN: 1067-1927
Publication date: 20011100
Publisher: BLACKWELL PUBLISHING INC, 350 MAIN ST, MALDEN, MA 02148 USA Language: English
Document Type: ARTICLE
Abstract: The expression of SPARC (secreted protein acidic and rich in *cysteine*/osteonectin/BM-40) is elevated in endothelial cells participating in angiogenesis in vitro and in vivo, SPARC acts on endothelial cells to elicit changes in cell shape and to inhibit cell cycle progression. In addition, SPARC binds to and diminishes the mitotic activity of vascular endothelial growth factor. To determine the effect(s) of SPARC on angiogenic responses in vivo, we implanted polyvinyl alcohol sponges subcutaneously into wild-type and SPARC-null mice. On days 12 and 20 following implantation, SPARC-null mice showed increased cellular invasion of the sponges in comparison to wild-type mice. Areas of the sponge with the highest cell density exhibited the highest numbers of vascular profiles in both wild-type and SPARC-null animals. The endothelial component of the vessels was substantiated by immunoreactivity with three different markers specific for endothelial cells, Although sponges from SPARC-null relative to wild-type mice were populated by significantly more cells and blood vessels, an increase in the ratio of vascular to nonvascular cells was not apparent. No differences in the percentage of proliferating cells within the sponge were detected between wildtype and SPARC-null sections. However, elevated levels of vascular endothelial growth factor were associated with sponges from SPARC-null versus wild-type mice, An increase in vascular endothelial growth factor production was also observed in SPARC-null primary dermal fibroblasts relative to those of wild-type cells. In conclusion, we have shown that the fibrovascular invasion of polyvinyl alcohol sponges is enhanced in mice lacking SPARC, and we propose that increased levels of vascular endothelial growth factor account, at least in part, for this response.

7/3,AB/48 (Item 2 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
(c) 2002 Inst for Sci Info. All rts. reserv.

10018492 Genuine Article#: 476BE Number of References: 37 Title: Induction of SPARC by *VEGF* in human vascular endothelial cells (ABSTRACT AVAILABLE)
Author(s): Kato Y (REPRINT) ; Lewalle JM; Baba Y; Tsukuda M; Sakai N; Baba M; Kobayashi K; Koshika S; Nagashima Y; Frankenne F; Noel A; Foidart JM ; Hata RI
Corporate Source: Kanagawa Dent Coll,Dept Biochem & Mol Biol, Bioventure Res Ctr,82 Inaoka Cho/Yokosuka/Kanagawa 2388580/Japan/ (REPRINT); Kanagawa Dent Coll,Dept Biochem & Mol Biol, Bioventure Res Ctr,Yokosuka/Kanagawa 2388580/Japan/; Kanagawa Dent Coll,Div Gene Regulat, Bioventure Res Ctr,Yokosuka/Kanagawa 2388580/Japan/; Univ Liege,Fac Med, Lab Tumor & Dev Biol,B-4000 Liege//Belgium/; Yokohama City Univ,Sch Med, Dept Pathol,Yokohama/Kanagawa 2360004/Japan/; Kanagawa Dent Coll,Bioventure Res Ctr, Div Gene Regulat,Yokosuka/Kanagawa 2388580/Japan/
Journal: BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, 2001, V287, N2 (SEP 21), P422-426
ISSN: 0006-291X Publication date: 20010921
Publisher: ACADEMIC PRESS INC, 525 B ST, STE 1900, SAN DIEGO, CA 92101-4495 USA
Language: English Document Type: ARTICLE
Abstract: SPARC/osteonectin/BM-40 is a matricellular protein that is thought to be involved in angiogenesis and endothelial barrier function. Previously, we have detected high levels of SPARC expression in endothelial cells (ECs) adjacent to carcinomas of kidney and tongue. Although SPARC-derived peptide showed an angiogenic effect, intact SPARC itself inhibited the mitogenic activity of vascular endothelial growth factor (*VEGF*) for ECs by the inhibiting phosphorylation of flt-1 (*VEGF* receptor 1) and subsequent ERK activation. Thus, the role of SPARC in tumor angiogenesis, stimulation or inhibition, is still unclear. To clarify the role of SPARC in tumor growth and progression, we

determined the effect of *VEGF* on the expression of SPARC in human microvascular EC line, HMEC-1, and human umbilical vein ECs. *VEGF* increased the levels of SPARC protein and steady-state levels of SPARC mRNA in serum-starved HMEC-1 cells. Inhibitors (SE202190 and SB203580) of p38, a mitogen-activated protein (MAP) kinase, attenuated *VEGF*-stimulated SPARC production in ECs. Since intact SPARC inhibits phosphorylation ERK MAP kinase in *VEGF* signaling, it was suggested that SPARC plays a dual role in the *VEGF* functions, tumor angiogenesis, and extravasation of tumors mediated by the increased permeability of endothelial barrier function. (C) 2001 Academic Press.

7/3,AB/49 (Item 3 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
(c) 2002 Inst for Sci Info. All rts. reserv.

09579079 Genuine Article#: 421UK Number of References: 61 Title: The crystal structure of human placenta growth factor-1 (PlGF-1), an angiogenic protein, at 2.0 angstrom resolution (ABSTRACT AVAILABLE) Author(s): Iyer S; Leonidas DD; Swaminathan GJ; Maglione D; Battisti M; Tucci M; Persico MG; Acharya KR (REPRINT)
Corporate Source: Univ Bath,Dept Biol & Biochem,Claverton Down/Bath BA2 7AY/Avon/England/ (REPRINT); Univ Bath,Dept Biol & Biochem,Bath BA2 7AY/Avon/England/; Geymonat SpA,I-03012 Anagni/FR/Italy/; CNR,Int Inst Genet & Biophys,I-80125 Naples//Italy/
Journal: JOURNAL OF BIOLOGICAL CHEMISTRY, 2001, V276, N15 (APR 13), P 12153-12161
ISSN: 0021-9258 Publication date: 20010413
Publisher: AMER SOC BIOCHEMISTRY MOLECULAR BIOLOGY INC, 9650 ROCKVILLE PIKE, BETHESDA, MD 20814 USA
Language: English Document Type: ARTICLE
Abstract: The angiogenic molecule placenta growth factor (PlGF) is a member of the *cysteine*-knot family of growth factors. In this study, a mature isoform of the human PlGF protein, PlGF-1, was crystallized as a homodimer in the crystallographic asymmetric unit, and its crystal structure was elucidated at 2.0 Angstrom resolution. The overall structure of PlGF-1 is similar to that of vascular endothelial growth factor (*VEGF*) with which it shares 42% amino acid sequence identity. Based on structural and biochemical data, we have mapped several important residues on the PlGF-1 molecule that are involved in recognition of the fms-like tyrosine kinase receptor (Flt-1, also known as VEGFR-1). We propose a model for the association of PlGF-1 and Flt-1 domain 2 with precise shape complementarity, consider the relevance of this assembly for PlGF-1 signal transduction, and provide a structural basis for altered specificity of this molecule.

7/3,AB/50 (Item 4 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
(c) 2002 Inst for Sci Info. All rts. reserv.

09353197 Genuine Article#: 398EV Number of References: 52 Title: N-acetylcysteine downregulates vascular endothelial growth factor production by human keratinocytes in vitro (ABSTRACT AVAILABLE) Author(s): Redondo P (REPRINT) ; Jimenez E; Perez A; Garcia-Foncillas J Corporate Source: Univ Navarra Clin,Sch Med, Dept Dermatol,Pamplona 31080//Spain/ (REPRINT); Univ Navarra Clin,Sch Med, Dept Dermatol,Pamplona 31080//Spain/; Univ Navarra Clin,Sch Med, Dept Mol Biol,Pamplona//Spain/; Univ Navarra Clin,Sch Med, Dept Vasc Biol & Thrombosis,Pamplona//Spain/
Journal: ARCHIVES OF DERMATOLOGICAL RESEARCH, 2000, V292, N12 (DEC), P 621-628
ISSN: 0340-3696 Publication date: 20001200
Publisher: SPRINGER-VERLAG, 175 FIFTH AVE, NEW YORK, NY 10010 USA Language: English Document Type: ARTICLE
Abstract: The present study was designed to evaluate the action of various antioxidants including N-acetyl-*cysteine* (NAC) and the flavonoids resveratrol and quercetin on the production of *VEGF* by human keratinocytes (HKC). NAC, resveratrol, and quercetin dose-dependently suppressed the incorporation of H-3-thymidine into HKC. Values of median inhibitory concentration for NBC, resveratrol, and quercetin were 10 mM, 55 muM, and 15 muM, respectively (P < 0.01). RT-PCR demonstrated *VEGF* 121 and *VEGF* 206 expression in all HKC samples. HKC showed baseline expression and a progressive gradual time-dependent increase in *VEGF* secretion (510 +/- 75 pg/ml at 24 h), and EGF (2.5-100 ng/ml) enhanced the secretion of *VEGF* in a dose-dependent fashion. HKC were incubated with NAC (2.5-20 mM) for 2 h prior to the addition of EGF (5 ng/ml) or PMA (10 ng/ml), and a significant decrease (P < 0.01) was found after 24 h of incubation with 2.5 mM NAC. However, neither resveratrol nor quercetin reduced the synthesis of this cytokine. In summary we conclude that NAC and the flavonoid antioxidants resveratrol and quercetin inhibit HKC proliferation regardless of the stage of differentiation and that NAC significantly inhibits *VEGF* secretion in basal and EGF- or PMA-treated HKC.

7/3,AB/51 (Item 5 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
(c) 2002 Inst for Sci Info. All rts. reserv.

08550816 Genuine Article#: 299JK Number of References: 39 Title: Saturation of, and competition for entry into, the apical secretory pathway (ABSTRACT AVAILABLE)
Author(s): Marmorstein AD; Csaky KG; Baffi J; Lam L; Rahaal F; RodriguezBoulan E (REPRINT)
Corporate Source: CORNELL UNIV MED COLL,MARGARET M DYSON VIS RES INST, DEPT OPHTHALMOL, 1300 YORK AVE/NEW YORK//NY/10021 (REPRINT); CORNELL UNIV MED COLL,MARGARET M DYSON VIS RES INST, DEPT OPHTHALMOL/NEW YORK//NY/10021; CORNELL UNIV MED COLL,DEPT CELL BIOL/NEW YORK//NY/10021 ; NEI,IMMUNOL LAB/BETHESDA//MD/20892
Journal: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, 2000, V97, N7 (MAR 28), P3248-3253
ISSN: 0027-8424 Publication date: 20000328
Publisher: NATL ACAD SCIENCES, 2101 CONSTITUTION AVE NW, WASHINGTON, DC 20418
Language: English Document Type: ARTICLE
Abstract: To investigate mechanisms of apical sorting in the secretory pathway of epithelial cells, we expressed varying amounts of the 165 amino acid isoform of vascular endothelial growth factor (*VEGF* (165)) and transforming growth factor beta 1 (TGF-beta 1) via replication defective adenoviruses, Apical sorting of both proteins was efficient at low expression levels but saturated or was reversed at high expression levers. High expression levels of TGF-beta 1 were effective at competing *VEGF*(165) out of the apical pathway; however. *VEGF*(165) did not compete out TGF-beta 1. Tunicamycin inhibition experiments showed that the apical polarity of *VEGF* (165) was independent of N-glycosylation. We conclude that the apical sorting of these two molecules is a saturable, signal-mediated process, involving competition for apical sorting receptors. The sorting of the two proteins does not appear to involve N-glycans as sorting signals, or lectin sorters. The observations are particularly relevant to gene therapy because they demonstrate that overexpression of a transgene can result in undesirable missorting of the encoded protein.

7/3,AB/52 (Item 6 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
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07874822 Genuine Article#: 219LN Number of References: 19 Title: Structure and function of vascular endothelial growth factor and its related proteins (ABSTRACT AVAILABLE)
Author(s): Pan X; Cao GW; Ke CW; He X; Qi ZT
Corporate Source: MIL MED COLL 2,DEPT MICROBIOL/SHANGHAI 200433//PEOPLES R CHINA/
Journal: ACTA BIOCHIMICA ET BIOPHYSICA SINICA, 1999, V31, N4 (JUL), P 357-361
ISSN: 0582-9879 Publication date: 19990700
Publisher: SHANGHAI INST BIOCHEMISTRY, ACADEMIA SINICA, 320 YUE-YANG ROAD, SHANGHAI 20031, PEOPLES R CHINA
Language: Chinese Document Type: REVIEW
Abstract: Vascular endothelial growth factor(*VEGF*) is a highly specific mitogen promoting the formation of blood vessels in embryogenesis and wound healing. It is also a potent inducer of vascular permeability. It is a member of the *cystine* knot growth factor superfamily. A detailed structural and functional characterization of the interactions between *VEGF* and its receptors is a prerequisite for the design of molecule antagonists. These structural characterizations and biological properties make *VEGF* an important research object in the fields of neovascularization of ischemia tissues, prognosis of cancers, tumor metastasis, and the gene therapy.

7/3,AB/53 (Item 7 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
(c) 2002 Inst for Sci Info. All rts. reserv.

06263749 Genuine Article#: YF024 Number of References: 67 Title: The crystal structure of vascular endothelial growth factor (*VEGF*) refined to 1.93 angstrom resolution: multiple copy flexibility and receptor binding (ABSTRACT AVAILABLE) Author(s): Muller YA; Christinger HW; Keyt BA; deVos AM (REPRINT) Corporate Source: GENENTECH INC,DEPT PROT ENGN, 460 POINT SAN BRUNO BLVD/S SAN FRANCISCO//CA/94080 (REPRINT); GENENTECH INC,DEPT PROT ENGN/S SAN FRANCISCO//CA/94080; GENENTECH INC,DEPT CARDIOVASC RES/S SAN FRANCISCO//CA/94080
Journal: STRUCTURE, 1997, V5, N10 (OCT 15), P1325-1338
ISSN: 0969-2126 Publication date: 19971015
Publisher: CURRENT BIOLOGY LTD, 34-42 CLEVELAND STREET, LONDON, ENGLAND W1P 6LB
Language: English Document Type: ARTICLE
Abstract: Background: Vascular endothelial growth factor (*VEGF*) is an endothelial cell-specific angiogenic and vasculogenic mitogen. *VEGF* also plays a role in pathogenic vascularization which is associated with a number of clinical disorders, including cancer and rheumatoid arthritis. The development of *VEGF* antagonists, which prevent the interaction of *VEGF* with its receptor, may be important for the treatment of such disorders. *VEGF* is a homodimeric member of the *cystine* knot growth factor superfamily, showing greatest similarity to platelet-derived growth factor

(PDGF). *VEGF* binds to two different tyrosine kinase receptors, kinase domain receptor (KDR) and Fms-like tyrosine kinase 1 (Flt-1), and a number of *VEGF* homologs are known with distinct patterns of specificity for these same receptors. The structure of *VEGF* will help define the location of the receptor-binding site, and shed light on the differences in specificity and cross-reactivity among the *VEGF* homologs.

Results: We have determined the crystal structure of the receptor-binding domain of *VEGF* at 1.93 Angstrom resolution in a triclinic space group containing eight monomers in the asymmetric unit. Superposition of the eight copies of *VEGF* shows that the beta-sheet core regions of the monomers are very similar, with slightly greater differences in most loop regions, For one loop, the different copies represent different snapshots of a concerted motion. Mutagenesis mapping shows that this loop is part of the receptor-binding site of *VEGF*.

Conclusions: A comparison of the eight independent copies of *VEGF* in the asymmetric unit indicates the conformational space sampled by the protein in solution; the root mean square differences observed are similar to those seen in ensembles of the highest precision NMR structures. Mapping the receptor-binding determinants on a multiple sequence alignment of *VEGF* homologs, suggests the differences in specificity towards KDR and Flt-1 may derive from both sequence variation and changes in the flexibility of binding loops. The structure can also be used to predict possible receptor-binding determinants for related *cystine* knot growth factors, such as PDGF.

7/3,AB/54 (Item 8 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
(c) 2002 Inst for Sci Info. All rts. reserv.

03633696 Genuine Article#: PU866 Number of References: 38 Title: VASCULAR ENDOTHELIAL GROWTH-FACTOR IN OCULAR FLUID OF PATIENTS WITH DIABETIC-RETINOPATHY AND OTHER RETINAL DISORDERS (Abstract Available) Author(s): AIELLO LP; AVERY RL; ARRIGG PG; KEYT BA; JAMPEL HD; SHAH ST; PASQUALE LR; THIEME H; IWAMOTO MA; PARK JE; NGUYEN HV; AIELLO LM; FERRARA N; KING GL
Corporate Source: JOSLIN DIABET CTR,BEETHAM EYE INST,DEPT OPHTHALMOL,1 JOSLIN PL/BOSTON//MA/02215; JOSLIN DIABET CTR,DIV RES/BOSTON//MA/02215; BRIGHAM & WOMENS HOSP,DEPT MED/BOSTON//MA/02115; BRIGHAM & WOMENS HOSP,DEPT OPHTHALMOL/BOSTON//MA/00000; HARVARD UNIV,SCH MED/BOSTON//MA/00000; UNIV CALIF SANTA BARBARA,NEUROSCI RES INST/SANTA BARBARA//CA/93106; GENENTECH INC/SAN FRANCISCO//CA/00000; JOHNS HOPKINS UNIV HOSP,WILMER OPHTHALMOL INST,DEPT OPHTHALMOL/BALTIMORE//MD/00000 Journal: NEW ENGLAND JOURNAL OF MEDICINE, 1994, V331, N22 (DEC 1), P 1480-1487
ISSN: 0028-4793
Language: ENGLISH Document Type: ARTICLE
Abstract: Background. Retinal ischemia induces intraocular neovascularization, which often leads to glaucoma, vitreous hemorrhage, and retinal detachment, presumably by stimulating the release of angiogenic molecules. Vascular endothelial growth factor (*VEGF*) is an endothelial-cell-specific angiogenic factor whose production is increased by hypoxia.

Methods. We measured the concentration of *VEGF* in 210 specimens of ocular fluid obtained from 164 patients undergoing intraocular surgery, using both radioimmunoassays and radioreceptor assays. Vitreous proliferative potential was measured with in vitro assays of the growth of retinal endothelial cells and with *VEGF* -neutralizing antibody.

Results. *VEGF* was detected in 69 of 136 ocular-fluid samples from patients with diabetic retinopathy, 29 of 38 samples from patients with neovascularization of the iris, and 3 of 4 samples from patients with ischemic occlusion of the central retinal vein, as compared with 2 of 31 samples from patients with no neovascular disorders ($P<0.001$, $P<0.001$, and $P = 0.006$, respectively). The mean (+/-SD) *VEGF* concentration in 70 samples of ocular fluid from patients with active proliferative diabetic retinopathy (3.6+/-6.3 ng per milliliter) was higher than that in 25 samples from patients with nonproliferative diabetic retinopathy (0.1+/-0.1 ng per milliliter, $P = 0.008$), 41 samples from patients with quiescent proliferative diabetic retinopathy (0.2+/-0.6 ng per milliliter, $P<0.001$), or 31 samples from nondiabetic patients (0.1+/-0.2 ng per milliliter, $P = 0.003$). Concentrations of *VEGF* in vitreous fluid (8.8+/-9.9 ng per milliliter) were higher than those in aqueous fluid (5.6+/-8.6 ng per milliliter, $P = 0.033$) in all 10 pairs of samples obtained simultaneously from the same patient; *VEGF* concentrations in vitreous fluid declined after successful laser photocoagulation. *VEGF* stimulated the growth of retinal endothelial cells in vitro, as did vitreous fluid containing measurable *VEGF*. Stimulation was inhibited by *VEGF*-neutralizing antibodies.

Conclusions. Our data suggest that *VEGF* plays a major part in mediating active intraocular

neovascularization in patients with ischemic retinal diseases, such as diabetic retinopathy and retinal-vein occlusion.

7/3,AB/55 (Item 9 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
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03633082 Genuine Article#: PT392 Number of References: 55 Title: SIGNALING PROPERTIES OF FLT4, A PROTEOLYTICALLY PROCESSED RECEPTOR TYROSINE KINASE RELATED TO 2 *VEGF* RECEPTORS (Abstract Available) Author(s): PAJUSOLA K; APRELIKOVA O; PELICCI G; WEICH H; CLAESSONWELSH L; ALITALO K
Corporate Source: UNIV HELSINKI,DEPT PATHOL,MOLEC CANC BIOL LAB,PL21/SF-00014 HELSINKI//FINLAND/; UNIV HELSINKI,DEPT PATHOL,MOLEC CANC BIOL LAB/SF-00014 HELSINKI//FINLAND/; UNIV PERUGIA,MONTELUCE POLICLIN,IST CLIN MED/I-06100 PERUGIA//ITALY/; GESELL BIOTECHNOL FORSCH MBH,DEPT GENE EXPRESS/W-3300 BRAUNSCHWEIG//GERMANY/; LUDWIG INST CANC RES,UPPSALA BRANCH/S-75124 UPPSALA//SWEDEN/
Journal: ONCOGENE, 1994, V9, N12 (DEC), P3545-3555
ISSN: 0950-9232
Language: ENGLISH Document Type: ARTICLE
Abstract: The FLT4, FLT1 and KDR/FLK1 genes encode, structurally similar endothelial cell receptor tyrosine kinases. Recently it has been shown that the FLT1 and KDR/FLK-1 proteins function as high-affinity receptors for vascular endothelial growth factor (*VEGF*). Here we show that FLT4 does not act as a receptor for *VEGF*, as *VEGF* did not show specific binding to the FLT4 tyrosine kinase or induce its autophosphorylation. Also, FLT4 did not interact with KDR in response to *VEGF*. However, when fused with the ligand binding domain of the colony stimulating factor-1 receptor (CSF-1R), the FLT4 tyrosine kinase was specifically activated by CSF-1. The activated FLT4 tyrosine kinase domain was found to interact with the Src homology 2 domains of the SHC and GRB2 adaptor proteins in vitro and with SHC in cells. CSF-1 stimulation of the CSF-1R/FLT4 receptor chimera induced thymidine incorporation in serum-starved NIH3T3 fibroblasts, but not in porcine aortic or murine lung capillary endothelial cells, although tyrosyl phosphorylation of the receptor and SHC occurred in these cells as well. These results suggest that the endothelial cell FLT4 receptor tyrosine kinase transmits signals for an as yet unidentified growth factor.

7/3,AB/56 (Item 10 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
(c) 2002 Inst for Sci Info. All rts. reserv.

03552619 Genuine Article#: PM044 Number of References: 22 Title: ADENOSINE AS AN ENDOGENOUS MEDIATOR OF HYPOXIA FOR INDUCTION OF VASCULAR ENDOTHELIAL GROWTH-FACTOR MESSENGER-RNA IN U-937 CELLS (Abstract Available)
Author(s): HASHIMOTO E; KAGE K; OGITA T; NAKAOKA T; MATSUOKA R; KIRA Y Corporate Source: UNIV TOKYO,BRANCH HOSP,DEPT INTERNAL MED 4,CARDIOVASC RES LAB,BUNKYO KU,3-28-6 MEJIRODAI/TOKYO 112//JAPAN/; TOKYO WOMENS MED COLL/TOKYO 162//JAPAN/; SHOWA GEN HOSP/KODAIRA/TOKYO/JAPAN/ Journal: BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, 1994, V204, N1 (OCT 14), P318-324
ISSN: 0006-291X
Language: ENGLISH Document Type: ARTICLE
Abstract: Adenosine induced by hypoxia exerts Various effects via different types of receptors. Recently, hypoxia was shown to be a strong inducer of vascular endothelial growth factor, a secreted endothelial cell specific mitogen. In this report, we studied on effects of adenosine on inducibility of *VEGF* and possible mediation of hypoxia for its induction in U-937 cells. Hypoxia induced expression of *VEGF* mRNA with an early peak at 1 hour. 5'-N-ethylcarboxamidoadenosine, an adenosine analog, strongly induced *VEGF* mRNA, which was inhibited by 3,7-dimethyl-1-propargylxanthine (DMPX), an A2-antagonist. The hypoxic induction was inhibited by adenosine deaminase, 7-(beta-hydroxyethyl)theophyline, a non-selective adenosine receptor antagonist and DMPX. These results suggest that the hypoxic induction of *VEGF* mRNA is mediated by adenosine via At-receptor in U-937 cells. (C) 1994 Academic Press, Inc.

7/3,AB/57 (Item 11 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
(c) 2002 Inst for Sci Info. All rts. reserv.

03482937 Genuine Article#: PG801 Number of References: 28 Title: VARIATIONS IN THE SIZE AND SULFATION OF HEPARIN MODULATE THE EFFECT OF HEPARIN ON THE BINDING OF *VEGF*(165) TO ITS RECEPTORS (Abstract Available)
Author(s): SOKER S; GOLDSTAUB D; SVAHN CM; VLODAVSKY I; LEVI BZ; NEUFELD G Corporate Source: TECHNION ISRAEL INST TECHNOL,DEPT BIOL/IL-32000HAIFA//ISRAEL/; TECHNION ISRAEL INST TECHNOL,DEPT BIOL/IL-32000HAIFA//ISRAEL/; TECHNION ISRAEL

INST TECHNOL,DEPT FOOD ENGN & BIOTECHNOL/IL-32000 HAIFA//ISRAEL/; HADASSAH UNIV HOSP,DEPT ONCOL/IL-91120 JERUSALEM//ISRAEL/; KABI PHARM THERAPEUT,DIV RES & DEV/S-11287 STOCKHOLM//SWEDEN/
Journal: BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, 1994, V203, N2 (SEP 15), P1339-1347
ISSN: 0006-291X
Language: ENGLISH Document Type: ARTICLE
Abstract: The binding of the 165 amino-acid form of vascular endothelial growth factor (*VEGF*(165)) to the *VEGF* receptors of vascular endothelial cells was potentiated by heparin and heparan-sulfate, but not by other glycosaminoglycans. Heparin fragments of 16-18 sugar units inhibited the binding of I-125-*VEGF*(165) to *VEGF* receptors, while fragments larger than 22 sugar units potentiated the binding. Over-sulfated heparin was a better potentiator of I-125-*VEGF*(165) binding than native heparin. O-desulfated and N-desulfated heparins potentiated the binding to a lesser extent than native heparin. Heparin and N-desulfated heparin efficiently inhibited the binding of I-125- *VEGF*(165) to alpha 2-macroglobulin, but surprisingly, O-desulfated heparin was an ineffective inhibitor. Since alpha 2-macroglobulin does not bind heparin, it follows that *VEGF*(165) does not bind O-desulfated heparin efficiently. These results suggest that the mechanism by which heparin modulates the binding of *VEGF* (165) to the *VEGF* receptors may require an interaction with cell surface heparin binding molecules. (C) 1994 Academic Press, Inc.

7/3,AB/58 (Item 12 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
(c) 2002 Inst for Sci Info. All rts. reserv.

03225203 Genuine Article#: NN525 Number of References: 63 Title: EXPRESSION OF PROTEIN-TYROSINE KINASES IN ISLET CELLS - POSSIBLE ROLE OF THE FLK-1 RECEPTOR FOR BETA-CELL MATURATION FROM DUCT CELLS (Abstract Available)
Author(s): OBERG C; WALTENBERGER J; CLAESSONWELSH L; WELSH M Corporate Source: UNIV UPPSALA,BIOMED CTR,DEPT MED CELL BIOL,BOX 571/S-75123 UPPSALA//SWEDEN/; UNIV UPPSALA,BIOMED CTR,DEPT MED CELL BIOL/S-75123 UPPSALA//SWEDEN/; LUDWIG INST CANC RES,CTR BIOMED,UPPSALA BRANCH/S-75124 UPPSALA//SWEDEN/; UNIV ULM,MED CTR,DEPT MED 2/W-7900 ULM//GERMANY/
Journal: GROWTH FACTORS, 1994, V10, N2, P115-126
ISSN: 0897-7194
Language: ENGLISH Document Type: ARTICLE
Abstract: To elucidate the expression of genes of importance for beta-cell replication and the production of insulin, single-stranded cDNAs from different preparations of insulin producing cells were used as template for the polymerase chain reaction (PCR) using primers specific for protein tyrosine kinases (PTKs). In RINm5F cells, as well as in fetal rat islets, the receptor PTK fetal liver kinase-1 (Flk-1) was expressed among other receptor and cytoplasmic tyrosine kinases. To elucidate the putative effects of stimulation of the Flk-1 receptor, fetal rat islet-like structures were cultured in the presence of the ligand for this receptor, vascular endothelial growth factor (*VEGF*). *VEGF* was found to stimulate both the insulin content/ islet DNA ratio and the accumulation of insulin in the culture medium without affecting the rates of beta-cell replication. To investigate the localization of expression of the Flk-1 receptor in the pancreas, serial sections of fetal pancreata were immunostained for Flk-1 and insulin. Expression of Flk-1 was detected in endothelial-like cells and cells lining pancreatic ducts. The latter are considered to contain precursor cells for the endocrine pancreas. In conclusion, specific protein tyrosine kinases are expressed in islet cells, and are presumably participating in the regulation of islet function. Specifically, the receptor PTK Flk-1 may play a role of beta-cell maturation from pancreatic duct cells.

7/3,AB/59 (Item 13 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
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03101653 Genuine Article#: NF150 Number of References: 47 Title: STABLE AND TEMPERATURE-SENSITIVE TRANSFORMATION OF RAT-KIDNEY EPITHELIAL-CELLS SUPPRESSES EXPRESSION OF ACIDIC FIBROBLAST GROWTH-FACTOR-1 BUT ACTIVATES SECRETION OF FIBROBLAST GROWTH-FACTOR-3 (INT-2) AND VASCULAR ENDOTHELIAL GROWTH-FACTOR (Abstract Available) Author(s): ZHANG GH; SATO JD; HERLEY MT; TSANG MWK; YE H; LIU H; ICHIMURA T ; YAN GC; MCKEEHAN WL; STEVENS JL
Corporate Source: W ALTON JONES CELL SCI CTR,10 OLD BARN RD/LAKE PLACID//NY/12946; W ALTON JONES CELL SCI CTR/LAKE PLACID//NY/12946
Journal: CELL GROWTH & DIFFERENTIATION, 1994, V5, N4 (APR), P349-357 ISSN: 1044-9523
Language: ENGLISH Document Type: ARTICLE
Abstract: Rat kidney proximal tubule epithelial cells (RPTE) in primary culture express acidic fibroblast growth factor 1 (FGF-1). Transformation of RPTE by SV40 (SV-RPTE) suppressed FGF-1 expression but activated secretion of FGF-like factor(s). SV-RPTE conditioned medium contained growth-promoting

activity for SV-RPTE and human umbilical vein endothelial cells, indicating that both autocrine and angiogenic factors were secreted. Reverse transcriptase-polymerase chain reaction and Northern analysis for various FCFs showed that only FGF-3, also known as int-2 mRNA was expressed in SV-RPTE. In addition, expression of mRNA for the heparin-binding angiogenic factor vascular endothelial growth factor (*VEGF*) increased dramatically in SV-RPTE. Physical characterization of the activity in the SV-RPTE conditioned medium suggested that FGF-3 and *VEGF* contributed the autocrine and angiogenic activities, respectively. We also investigated FGF-3 and *VEGF* secretion in temperature-sensitive (ts) SV40-transformed RPTE. tsSV-RPTE had transformed properties resembling those of SV-RPTE only at the permissive temperature (33 degrees C), e.g., increased growth potential and anchorage-independent growth. FGF-1 was expressed only at the nonpermissive temperature. *VEGF* mRNA levels and secretion of the human umbilical vein endothelial cell growth-promoting activity were reduced by switching tsSV-RPTE cells from 33 degrees to 39 degrees C. However, FGF-3 mRNA levels were not affected significantly by the temperature switch suggesting that activation of *VEGF* and FGF-3 occurs through different mechanisms. These results indicate that FGF-1 expression in RPTE is suppressed by SV40 transformation. However, secretion of the angiogenic factor *VEGF* and formation of a new FGF-like autocrine loop through activation of FGF-3 expression may contribute to the transformation of renal epithelial cells.

7/3,AB/60 (Item 14 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
(c) 2002 Inst for Sci Info. All rts. reserv.

03053096 Genuine Article#: MZ645 Number of References: 211 Title: MACROPHAGES AND ANGIOGENESIS (Abstract Available)
Author(s): SUNDERKOTTER C; STEINBRINK K; GOEBELER M; BHARDWAJ R; SORG C Corporate Source: UNIV MUNSTER,DEPT DERMATOL,VON ESMARCH STR 56/D-48149 MUNSTER//GERMANY/; UNIV MUNSTER,INST EXPTL DERMATOL/W-4400 MUNSTER//GERMANY/
Journal: JOURNAL OF LEUKOCYTE BIOLOGY, 1994, V55, N3 (MAR), P410-422 ISSN: 0741-5400
Language: ENGLISH Document Type: REVIEW
Abstract: Macrophages are supposed to play a key role in inflammatory and tumor angiogenesis. Their importance derives from (1) their ubiquitous presence in normal and especially inflamed tissues, (2) their potential to become activated in response to appropriate stimuli, and (3) their repertoire of secretory products. By release of proteases, growth factors (bFGF, GM-CSF, TGF-cr, IGF-I, PDGF, *VEGF*/VPF, TGF-beta), and other monokines (IL-1, IL-6, IL-8, TNF-alpha, substance P, prostaglandins, interferons, thrombospondin 1), activated macrophages have the capability to influence each phase of the angiogenic process, such as alterations of the local extracellular matrix, induction of endothelial cells to migrate or proliferate, and inhibition of vascular growth with formation of differentiated capillaries. This review describes macrophage physiology and the influence of macrophage secretory products on the different phases of angiogenesis in vitro and in vivo.

7/3,AB/61 (Item 15 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
(c) 2002 Inst for Sci Info. All rts. reserv.

03004546 Genuine Article#: MZ503 Number of References: 38 Title: VASCULAR ENDOTHELIAL GROWTH-FACTOR IS INDUCED IN RESPONSE TO TRANSFORMING GROWTH-FACTOR-BETA IN FIBROBLASTIC AND EPITHELIAL-CELLS (Abstract Available)
Author(s): PERTOVAARA L; KAIPAINEN A; MUSTONEN T; ORPANA A; FERRARA N; SAKSELA O; ALITALO K Corporate Source: UNIV HELSINKI,DEPT PATHOL,MOLEC CANC BIOL LAB,HAARTMANINKATU 3/SF-00290 HELSINKI 29//FINLAND/; UNIV HELSINKI,DEPT PATHOL,MOLEC CANC BIOL LAB/SF-00290 HELSINKI 29//FINLAND/; UNIV HELSINKI,DEPT VIROL/SF-00290 HELSINKI 29//FINLAND/; UNIV HELSINKI,DEPT OBSTET & GYNECOL/SF-00290 HELSINKI 29//FINLAND/; GENENTECH INC/S SAN FRANCISCO//CA/94080
Journal: JOURNAL OF BIOLOGICAL CHEMISTRY, 1994, V269, N9 (MAR 4), P 6271-6274
ISSN: 0021-9258
Language: ENGLISH Document Type: NOTE
Abstract: Transforming growth factor beta (TGF-beta) is a multifunctional polypeptide that regulates the proliferation and differentiation of various cells and has an angiogenic effect in vivo although it inhibits the growth of cultured endothelial cells. We report here that TGF-beta treatment of quiescent cultures of mouse embryo-derived AKR-2B cells, which are growth-stimulated by TGF-beta, and human lung adenocarcinoma A549 cells, which are growth-inhibited by TGF-beta, results in the induction of vascular endothelial growth factor (*VEGF*) mRNA and protein. Maximal *VEGF* mRNA levels occurred 4-8 h after stimulation with a decline to background levels in 24 h. In contrast, the related placenta growth factor mRNA was not induced by TGF-beta in these cells. No *VEGF*

receptor mRNA was seen in AKR SB cells. Also, TGF-beta treatment of endothelial cells, which express the FLT1 and KDR/FLK-1 receptors for *VEGF*, did not cause *VEGF* induction. Because *VEGF* is known to be a strong angiogenic factor for endothelial cells, the results suggest that the angiogenic effect of TGF-beta on endothelial cells in blood vessels may be mediated at least partly by a paracrine induction of *VEGF* in other surrounding cell types.

7/3,AB/62 (Item 16 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
(c) 2002 Inst for Sci Info. All rts. reserv.

02991037 Genuine Article#: MU119 Number of References: 37 Title: A HEPARIN-BINDING FORM OF PLACENTA GROWTH-FACTOR (PLGF-2) IS EXPRESSED IN HUMAN UMBILICAL VEIN ENDOTHELIAL-CELLS AND IN PLACENTA (Abstract Available)
Author(s): HAUSER S; WEICH HA
Corporate Source: GESELL BIOTECHNOL FORSCH MBH,DEPT GENE EXPRESS/D-38124 BRAUNSCHWEIG//GERMANY/; GESELL BIOTECHNOL FORSCH MBH,DEPT GENE EXPRESS/D-38124 BRAUNSCHWEIG//GERMANY/; UNIV FREIBURG,INST MOLEC CELL BIOL/D-79108 FREIBURG//GERMANY/
Journal: GROWTH FACTORS, 1993, V9, N4, P259-268 ISSN: 0897-7194
Language: ENGLISH Document Type: ARTICLE
Abstract: Placenta Growth Factor (PlGF) was recently discovered as a secreted growth factor for vascular endothelial cells and based on its homology to vascular endothelial growth factor (*VEGF*), can be classified as a new member of this growth factor family. We have carried out polymerase chain amplification (PCR) of RNA from human umbilical vein endothelial cells and placenta tissue and discovered a second species of PlGF, PlGF-2. PlGF-2 has a 21-amino acid insertion not present in PlGF-1 coding for a highly basic region near the C-terminus. This is similar to VEGF189. Northern analysis has shown, that the PlGF gene is expressed only in a limited number of cell types and tissues, e.g. human umbilical vein endothelial cells (HUVE) and placenta. Infection of Sf158 insect cells with recombinant baculoviruses specific for the two forms showed, that both, PlGF-1 and PlGF-2 are secreted efficiently into the supernatant and PlGF-2 can bind with high affinity to heparin. Both PlGF forms had a similar mitog enic potency for bovine aortic endothelial cells. Binding studies with I-125-*VEGF*(165) demonstrate, that supernatant of PlGF expressing insect cells can compete for receptor binding. Similar to *VEGF*, PlGF can exist in different forms which are probably generated by differential splicing. The occurrence of two molecular forms of this endothelial specific growth factor suggests different physiological roles of the two forms during placental development and differentiation.

7/3,AB/63 (Item 17 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
(c) 2002 Inst for Sci Info. All rts. reserv.

02972378 Genuine Article#: MU413 Number of References: 48 Title: VASCULAR ENDOTHELIAL GROWTH-FACTOR (*VEGF*) ISOFORMS - DIFFERENTIAL DEPOSITION INTO THE SUBEPITHELIAL EXTRACELLULAR-MATRIX AND BIOACTIVITY OF EXTRACELLULAR MATRIX-BOUND *VEGF* (Abstract Available)
Author(s): PARK JE; KELLER GA; FERRARA N
Corporate Source: GENENTECH INC/S SAN FRANCISCO//CA/94080; GENENTECH INC/S SAN FRANCISCO//CA/94080
Journal: MOLECULAR BIOLOGY OF THE CELL, 1993, V4, N12 (DEC), P1317-1326 ISSN: 1059-1524
Language: ENGLISH Document Type: ARTICLE
Abstract: Vascular endothelial growth factor (*VEGF*)mRNA undergoes alternative splicing events that generate four different homodimeric isoforms, *VEGF*(121), *VEGF*(165), *VEGF*(189), Or *VEGF*(206). *VEGF*(121) is a nonheparin-binding acidic protein, which is freely diffusible. The longer forms, *VEGF*(189) or *VEGF*(206), are highly basic proteins tightly bound to extracellular heparin-containing proteoglycans. *VEGF*(165) has intermediate properties. To determine the localization of *VEGF* isoforms, transfected human embryonic kidney CEN4 cells expressing *VEGF*(165), *VEGF*(189), or *VEGF*(206) were stained by immunofluorescence with a specific monoclonal antibody. The staining was found in patches and streaks suggestive of extracellular matrix (ECM). *VEGF*(165) was observed largely in Golgi apparatus-like structures. Immunogold labeling of cells expressing *VEGF*(189) or *VEGF*(206) revealed that the staining was localized to the subepithelial ECM. *VEGF* associated with the ECM was bioactive, because endothelial cells cultured on ECM derived from cells expressing *VEGF*(189) or *VEGF*(206) were markedly stimulated to proliferate. In addition, ECM-bound *VEGF* can be released into a soluble and bioactive form by heparin or plasmin. ECM-bound *VEGF* (189) and *VEGF*(206) have molecular masses consistent with the intact polypeptides. The ECM may represent an important source of *VEGF* and angiogenic potential.

7/3,AB/64 (Item 18 from file: 34)

DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
(c) 2002 Inst for Sci Info. All rts. reserv.

02956611 Genuine Article#: MR912 Number of References: 35 Title: PARTIAL CHARACTERIZATION OF GLIOMA-DERIVED GROWTH-FACTOR-2 - A NOVEL MITOGENIC ACTIVITY FROM HUMAN CELL-LINE D-54 MG (Abstract Available) Author(s): LYON E; GILLESPIE GY
Corporate Source: UNIV ALABAMA,DIV NEUROL SURG,BRAIN TUMOR RES LABS,THT 65/BIRMINGHAM//AL/35294; UNIV ALABAMA,DIV NEUROL SURG,BRAIN TUMOR RES LABS/BIRMINGHAM//AL/35294
Journal: JOURNAL OF NEURO-ONCOLOGY, 1993, V17, N2, P99-109 ISSN: 0167-594X
Language: ENGLISH Document Type: ARTICLE
Abstract: We have shown that several human malignant glioma cell lines are stimulated by bacterial lipopolysaccharide (E. coil 0111:B4, 1 mu g/ml) to produce a high molecular weight (> 200 kD) growth activity for BALB 3T3, clone A31 cells [1,2]. This glioma-derived growth factor (GDGF-2) acts like a 'competence' factor. Malignant glioma cell line D-54 MG constitutively produced GDGF-2, which we have partially characterized from serum-free conditioned culture medium. GDGF-2 is resistant to heat (100 degrees C, 5 min), acidic (pH 2, 2 hr) or reducing (0.5 M 2 ME, 30 min) conditions as well as exposure to RNases; however, it is sensitive to > 4 freeze-thaw cycles, alkaline (pH 11, 2 hr) conditions or pre-treatment with proteolytic enzymes. GDGF-2 had a pi of 6.8 determined by preparative isoelectric focusing, bound to DEAF, with elution at 35 and 185 mM NaCl and at 43% acetonitrile from a C4 reversed phase column. GDGF-2 activity was not neutralized by antibodies to TGF alpha, TGF beta, PDGF, *VEGF* or TNF alpha indicating that it is not immunochemically related to these growth factors. However GDGF-2 co-chromatographed on Superose 12 HPLC (250 x 9 mm; 5% isopropanol, 6 mM CHAPS in PBS) with a substance that suppressed growth of mink lung epithelial cells (Mv1Lu), but not BALB 3T3 cells, and could be neutralized by anti-TGF beta antibodies. GDGF-2 activity eluted from heparin columns in 0.6 M NaCl; thus, it is not a heparin binding growth factor. D-54 MG cell line produced alpha(2)-macroglobulin (alpha(2)M), which is known to bind TGF beta; however, immunoprecipitation alpha(2)M did not deplete TGF beta or GDGF-2 activity. Further, neither GDGF-2 or TGF beta can be dissociated into lower molecular weight active components by chromatography in high salt (2 M NaCl) or 2-ME (0.5 M). GD GF-2 may be a novel autocrine or paracrine mitogen, stimulating mitotic division or interfering with normal cell growth regulation.

7/3,AB/65 (Item 19 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
(c) 2002 Inst for Sci Info. All rts. reserv.

02955168 Genuine Article#: MT022 Number of References: 57 Title: DIFFERENTIAL EXPRESSION OF THE 2 *VEGF* RECEPTORS FLT AND KDR IN PLACENTA AND VASCULAR ENDOTHELIAL-CELLS (Abstract Available) Author(s): BARLEON B; HAUSER S; SCHOLLMANN C; WEINDEL K; MARME D; YAYON A; WEICH HA
Corporate Source: GESELL BIOTECHNOL FORSCH MBH,DEPT GENE EXPRESS/D-38124 BRAUNSCHWEIG//GERMANY/; UNIV FREIBURG,INST MOLEC CELL BIOL/D-79108 FREIBURG//GERMANY/; WEIZMANN INST SCI,DEPT CHEM IMMUNOL/IL-76100 REHOVOT//ISRAEL/
Journal: JOURNAL OF CELLULAR BIOCHEMISTRY, 1994, V54, N1 (JAN), P56-66 ISSN: 0730-2312
Language: ENGLISH Document Type: ARTICLE
Abstract: Vascular endothelial growth factor (*VEGF*) is a newly identified growth and permeability factor with a unique specificity for endothelial cells. Recently the fit-encoded tyrosine kinase was characterized as a receptor for *VEGF*. A novel tyrosine kinase receptor encoded by the KDR gene was also found to bind *VEGF* with high affinity when expressed in CMT-3 cells. Screening for fit and KDR expression in a variety of species and tissue-derived endothelial cells demonstrates that fit is predominantly expressed in human placenta and human vascular endothelial cells. Placenta growth factor (PlGF), a growth factor significantly related to *VEGF*, is coexpressed with fit in placenta and human vascular endothelial cells. KDR is more widely distributed and expressed in all vessel-derived endothelial cells. These data demonstrate that cultured human endothelial cells isolated from different tissues express both *VEGF* receptors in relative high levels and, additionally, that all investigated nonhuman endothelial cells in culture are also positive for KDR gene expression. (C) 1994 Wiley-Liss, Inc.
? ds

Set Items Description
S1 50 VEGF AND (DISULFIDE OR DISULPHIDE)
S2 27 RD (unique items)
S3 0 S VEGF AND (VEGF OR VASCULAR)/TI
S4 11507 VEGF
S5 110 S4 AND (CYSTEINE OR CYSTINE)
S6 71 RD (unique items)
S7 65 S6 NOT S2
? s s4 and (dimer or dimeric) not (s2 or s7)
11507 S4
53419 DIMER
23352 DIMERIC

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        27  S2
        65  S7
   S8    92  S4 AND (DIMER OR DIMERIC) NOT (S2
OR S7) ? rd
...examined 50 records  (50)
...completed examining records
   S9    62  RD (unique items)
? s s9 not (s2 or s7
>>>Unmatched parentheses
? s s9 not (s2 or s7)
        62  S9
        27  S2
        65  S7
  S10    62  S9 NOT (S2 OR S7)
? t s10/3,ab/all

 10/3,AB/1    (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

13116379  21947953  PMID: 11950904
  Design of a variant of vascular endothelial growth
factor-A (*VEGF* -A) antagonizing KDR/Flk-1 and Flt-1.
  Leenders William; Lubsen Nicolette; van Altena
Margarethe; Clauss Matthias; Deckers Martine; Lowik
Clemens; Breier Georg; Ruiter Dirk; de Waal Robert
  Department of Pathology, University Medical Center
Nijmegen, The Netherlands. w.leenders@pathol.azn.nl
  Laboratory investigation; a journal of technical methods
and pathology ( United States)  Apr 2002, 82 (4)
p473-81, ISSN 0023-6837 Journal Code: 0376617
  Document type: Journal Article
  Languages: ENGLISH
  Main Citation Owner: NLM
  Record type: Completed
  Because of its central role in pathological
angiogenesis, vascular endothelial growth factor
(*VEGF* ) has become a major target for
anti-angiogenic therapies. We report here the
construction of a heterodimeric antagonistic *VEGF*
variant (HD-*VEGF* ). In this antagonist, binding
domains for the *VEGF*-receptors KDR/Flk-1 and Flt-1
are present at one pole of the *dimer*, whereas the
other pole carries domain swap mutations, which prevent
binding to either receptor. As HD-*VEGF* can only bind
to monomeric receptors, it does not lead to signal
transduction. Moreover, it antagonizes *VEGF* and
possibly other members of the *VEGF* family, which are
KDR/Flk-1 and Flt-1 ligands. We show here that
HD-*VEGF* is a potent inhibitor of *VEGF*-mediated
proliferation and tissue factor induction in endothelial
cell cultures, requiring only a 20-fold and a 4-fold
excess, respectively, to block the activity of wtVEGF
completely. A 4-fold excess of HD-*VEGF* over wtVEGF
was also sufficient to abrogate vascular permeability as
determined in the Miles assay in vivo. Furthermore,
HD-*VEGF* inhibited fetal bone angiogenesis in an ex
vivo assay. Thus, HD-*VEGF* blocks KDR- and
Flt-1-mediated *VEGF* activities that are crucial in the
angiogenic process and is therefore a promising,
multipotent compound in the treatment of
angiogenesis-related diseases.

 10/3,AB/2    (Item 2 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

13035880  21946619  PMID: 11950137
  Biology of erythropoietin.
  Jelkmann W; Hellwig-Burgel T
  Institute of Physiology, Medical University of Luebeck,
Germany.  Advances in experimental medicine and
biology (United States)  2001, 502 p169-87, ISSN
0065-2598  Journal Code: 0121103
  Document type: Journal Article
  Languages: ENGLISH
  Main Citation Owner: NLM
  Record type: In Process
  Hypoxia induces tissue-specific gene products such
as erythropoietin (EPO) and vascular endothelial growth
factor (*VEGF*), which improve the peripheral O2 supply,
and glucose transporters and glycolytic enzymes, which
adapt cells to reduced O2 availability. EPO has been the
fountainhead in research on pO2-dependent synthesis of
proteins. The EPO gene enhancer (like the flanking
DNA-elements of several other pO2-controlled genes)
contains a consensus sequence (CGTG) that binds
the trans-acting *dimeric* hypoxia-inducible factor
1 (HIF-1alpha/beta). The alpha-subunit of HIF-1 is
rapidly degraded by the proteasome under normoxic
conditions, but it is stabilized on occurrence of
hypoxia. HIF-1 DNA-binding is also increased by insulin,
and by interleukin-1 and tumor necrosis factor. Thus, in
some aspects there is synergy in the cellular responses
to hypoxia, glucose deficiency and inflammation. In
viewing clinical medicine recombinant human EPO
(rHu-EPO) has become the mainstay of treatment for
renal anemia. Endogenous EPO and rHu-EPO are similar
except for minor differences in the pattern of their 4
carbohydrate chains. RHu-EPO is also administered to
patients suffering from non-renal anemias, such as in
autoimmune diseases or malignancies. The correction of
anemia in patients with solid tumors is not merely
considered a palliative intervention. Hypoxia promotes
tumor growth. However, the benefits of the
administration of rHu-EPO to tumor patients with
respect to its positive effects on tumor oxygenation,
tumor growth inhibition and support of chemo- and
radiotherapy is still debatable ground.

 10/3,AB/3    (Item 3 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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13030355 21945423 PMID: 11948691
VEGF (165) mediates formation of complexes containing VEGFR-2 and neuropilin-1 that enhance *VEGF*(165)-receptor binding. Soker Shay; Miao Hua-Quan; Nomi Masashi; Takashima Seiji; Klagsbrun Michael
Department of Urology, Children's Hospital and Harvard Medical School, Boston, Massachusetts 02115.
Journal of cellular biochemistry (United States) 2002, 85 (2) p357-68, ISSN 0730-2312 Journal Code: 8205768
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: In Process
Co-expression of NRP1 and (VEGFR-2) KDR on the surface of endothelial cells (EC) enhances *VEGF* (165) binding to KDR and EC chemotaxis in response to *VEGF*(165). Overexpression of NRP1 by prostate tumor cells in vivo results in increased tumor angiogenesis and growth. We investigated the molecular mechanisms underlying NRP1-mediated angiogenesis by analyzing the association of NRP1 and KDR. An intracellular complex containing NRP1 and KDR was immunoprecipitated from EC by anti-NRP1 antibodies only in the presence of *VEGF*(165). In contrast, *VEGF*(121), which does not bind to NRP1, did not support complex formation. Complexes containing *VEGF*(165), NRP1, and KDR were also formed in an intercellular fashion by co-culture of EC expressing KDR only, with cells expressing NRP1 only, for example, breast carcinoma cells. *VEGF*(165) also mediated the binding of a soluble NRP1 *dimer* to cells expressing KDR only, confirming the formation of such complexes. Furthermore, the formation of complexes containing KDR and NRP1 markedly increased (125)I-*VEGF*(165) binding to KDR. Our results suggest that formation of a ternary complex of *VEGF*(165), KDR, and NRP1 potentiates *VEGF*(165) binding to KDR. These complexes are formed on the surface of EC and in a juxtacrine manner via association of tumor cell NRP1 and EC KDR. Copyright 2002 Wiley-Liss, Inc.

10/3,AB/4 (Item 4 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

12883350 21856773 PMID: 11866530
Solution Structure of a Phage-derived Peptide Antagonist in Complex with Vascular Endothelial Growth Factor.
Pan Borlan; Li Bing; Russell Stephen J; Tom Jeffrey Y K; Cochran Andrea G ; Fairbrother Wayne J
Department of Protein Engineering and
Journal of molecular biology (England) Feb 22 2002, 316 (3) p769-87, ISSN 0022-2836 Journal Code: 2985088R
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: In Process
Vascular endothelial growth factor (*VEGF*) is a potent endothelial cell-specific mediator of angiogenesis and vasculogenesis. *VEGF* is involved pathologically in cancer, proliferative retinopathy and rheumatoid arthritis, and as such represents an important therapeutic target. Three classes of disulfide-constrained peptides that antagonize binding of the *VEGF* *dimer* to its receptors, KDR and Flt-1, were identified previously using phage display methods. NMR studies of a representative peptide from the most potent class of these peptide antagonists, v107 (GGNECDAIRMWEWECFERL), were undertaken to characterize its interactions with *VEGF*. v107 has no defined structure free in solution, but binding to *VEGF* induces folding of the peptide. The solution structure of the *VEGF* receptor-binding domain-v107 complex was determined using 3940 (1970 per *VEGF* monomer) internuclear distance and 476 (238 per *VEGF* monomer) dihedral angle restraints derived from NMR data obtained using samples containing either (13)C/(15)N-labeled protein plus excess unlabeled peptide or (13)C/(15)N-labeled peptide plus excess unlabeled protein. Residual dipolar coupling restraints supplemented the structure determination of the complex and were found to increase significantly both the global precision of *VEGF* in the complex and the agreement with available crystal structures of *VEGF*. The calculated ensemble of structures is of high precision and is in excellent agreement with the experimental restraints. v107 has a turn-helix conformation with hydrophobic residues partitioned to one face of the peptide and polar or charged residues at the other face. Contacts between two v107 peptides and the *VEGF* *dimer* are mediated by primarily hydrophobic side-chain interactions. The v107-binding site on *VEGF* overlaps partially with the binding site of KDR and is similar to that for domain 2 of Flt-1. The structure of the *VEGF*-v107 complex provides new insight into how binding to *VEGF* can be achieved that may be useful for the design of small molecule antagonists. Copyright 2002 Elsevier Science Ltd.

10/3,AB/5 (Item 5 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

12750667 21634676 PMID: 11774259
VEGF expression and enhanced production by gonadotropins in ovarian epithelial tumors.
Wang Jun; Luo Feng; Lu Jean J; Chen Peter K; Liu Paul; Zheng Wenxin Department of Preventive Medicine, University of Southern California, Los Angeles, CA, USA.

International journal of cancer. Journal international du cancer (United States) Jan 10 2002, 97 (2) p163-7, ISSN 0020-7136 Journal Code: 0042124
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Vascular endothelial growth factor (*VEGF*) is a heparin-binding, *dimeric* polypeptide with potent mitogenic effects on endothelial cells. *VEGF* expression has also been reported in ovarian epithelial tumors (OETs), which may be associated with gonadotropin stimulatioin. We recently reported that most OETs, including OET cell lines, express gonadotropin receptors. Here we studied *VEGF* mRNA expression in 141 OET and 35 benign ovarian samples using reverse transcriptase polymerase chain reaction and in situ hybridization (ISH). We also studied *VEGF* production by OET cell lines under stimulation of gonadotropins. AO (serous carcinoma), low malignant potential (LMP; SV40-transformed borderline tumor) and ML-5 (SV40-transformed cystadenoma) cells were examined for *VEGF* protein production under the regulation of gonadotropins in vitro. The biologic function of *VEGF* was confirmed by using bovine endothelial growth assay. Whereas *VEGF* was not detected in benign ovarian surface epithelium or in ovarian epithelial inclusions, it was detected in both epithelial and stromal compartments of OETs. For *VEGF* epithelial expression, only 5% of ovarian cystadenomas and 30% of borderline tumors were positive for *VEGF* detection by ISH, whereas *VEGF* mRNA signal was detected in 80% of ovarian carcinoma cases. This increment of *VEGF* expression in ovarian carcinomas was statistically significant compared with benign and borderline tumors. Within ovarian carcinomas, the percentage of *VEGF*-positive cells was significantly associated with the grade of cancer but not with cancer cell types or cancer stages. Both follicle-stimulating hormone (FSH) and luteinizing hormone (LH) stimulated the expression of VEFG(165) in AO cells in a dose-dependent manner. Maximal induction was obtained for FSH at dose of 40 mIU/ml and for LH at 50 mIU/ml after 48 hr of culture. Compared with the nonstimulated cells, *VEGF* level was significantly elevated in both LMP and AO cells after stimulation of gonadotropins. Furthermore, the induction of *VEGF* expression was significantly stronger in carcinoma cells than in borderline OET cells. These observations suggest that *VEGF* may play a role in the development of ovarian cancer and that the elevated gonadotropins, as found in menopause and in most ovarian cancer patients after surgery, could accelerate tumor growth and tumor recurrence by inducing *VEGF* expression in OETs. Copyright 2002 Wiley-Liss, Inc.

10/3,AB/6 (Item 6 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

12711075 21621886 PMID: 11751375
A receptor for vascular endothelial growth factor that stimulates endothelial apoptosis.
Quinn T P; Soifer S J; Ramer K; Williams L T; Nakamura M C Cardiovascular Research Institute, University of California San Francisco, San Francisco, California 94143, USA. tpquinn@itsa.ucsf.edu Cancer research (United States) Dec 15 2001, 61 (24) p8629-37, ISSN 0008-5472 Journal Code: 2984705R
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Vascular endothelial growth factor (*VEGF*) is a *dimeric* angiogenic factor that is overexpressed by many tumors and stimulates tumor angiogenesis. *VEGF* initiates signaling by dimerizing the receptors VEGFR-1 and VEGFR-2. The Fas receptor stimulates apoptosis, and artificial dimerization of the Fas cytoplasmic domain has been shown to induce apoptosis. We constructed a chimeric receptor (VEGFR2Fas) combining the extracellular and transmembrane domains of VEGFR-2 with the cytoplasmic domain of Fas receptor. When VEGFR2Fas was stably expressed in endothelial cells in vitro, treatment with *VEGF* rapidly induced cell death with features characteristic of Fas-mediated apoptosis. These findings demonstrate that VEGFR2Fas functions as a *VEGF* -triggered death receptor and raise the possibility that introduction of VEGFR2Fas into tumor endothelium or tumor cells in vivo may convert tumor-derived *VEGF* from an angiogenic factor into an antiangiogenesis agent.

10/3,AB/7 (Item 7 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

11189160 21212042 PMID: 11312102
Signaling properties of *VEGF* receptor-1 and -2 homo- and heterodimers.
Huang K; Andersson C; Roomans G M; Ito N; Claesson-Welsh L Rudbeck Laboratory, Department of Genetics and Pathology, Uppsala University, S-751 85, Uppsala, Sweden.
international journal of biochemistry & cell biology (England) Apr 2001, 33 (4) p315-24, ISSN 1357-2725 Journal Code: 9508482 Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Vascular endothelial growth factor (*VEGF* -A) exerts its effects through receptor tyrosine kinases

VEGF receptor-1 (VEGFR-1) and VEGFR-2, which are expressed on most endothelial cell types in vitro and in vivo. We have examined *VEGF*-A-induced signal transduction in porcine aortic endothelial (PAE) cells individually expressing VEGFR-1 or VEGFR-2, and cells co-expressing both receptor types. We show that *VEGF* -A-stimulated PAE cells co-expressing VEGFR-1 and -2 contain receptor heterodimers. *VEGF*-A-stimulation of all three cell lines (expressing VEGFR-1, -2 and -1/2) resulted in signal transduction with different efficiencies. Thus, tyrosine phosphorylation of phospholipase Cgamma, and accumulation of inositol polyphosphates were efficiently transduced in the VEGFR-1/2 cells whereas cells expressing VEGFR-1 responded poorly in these assays. In contrast, *VEGF*-A-induced activation of phosphoinositide 3-kinase and induction of Ca2+ fluxes were transduced well by VEGFR-1 and VEGFR-2 homo- and heterodimers. The pattern of Ca2+ fluxes was unique for each type of *VEGF* receptor *dimer*. Our data show that signal transduction induced by *VEGF*-A is transduced in distinct manners by homo- and heterodimers of *VEGF* receptors.

10/3,AB/8 (Item 8 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

11188673 21211326 PMID: 11311231
A small peptide derived from Flt-1 (VEGFR-1) functions as an angiogenic inhibitor.
Tan D C; Kini R M; Jois S D; Lim D K; Xin L; Ge R
Department of Biological Sciences, National University of Singapore, Singapore 119260.
FEBS letters (Netherlands) Apr 13 2001, 494 (3) p150-6, ISSN 0014-5793 Journal Code: 0155157
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Vascular endothelial growth factor (*VEGF*) is an angiogenic stimulator which functions through two endothelial specific tyrosine kinase receptors, Flt-1 and Flk-1. In this work, we show that an 11-amino acid peptide derived from the second immunoglobulin-like domain of Flt-1 functions as an angiogenic inhibitor in chick chorioallantoic membrane and inhibited *VEGF*-induced vascular permeability in Miles' assay without binding to *VEGF* directly. Circular dichroism and nuclear magnetic resonance analyses indicate that this peptide forms a stable extended structure in solution, presumably beta-sheet structure and is most likely existing as a *dimer* . Our results suggest that this small peptide functions as an angiogenic inhibitor by inhibiting *VEGF* function through a non-*VEGF* binding mechanism.

10/3,AB/9 (Item 9 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

11164352 21193870 PMID: 11300596
Plasma vascular endothelial growth factor concentrations in healthy dogs and dogs with hemangiosarcoma.
Clifford C A; Hughes D; Beal M W; Mackin A J; Henry C J; Shofer F S; Sorenmo K U
University of Pennsylvania, Veterinary Teaching Hospital, Department of Clinical Sciences, School of Veterinary Medicine, Philadelphia 19104, USA. ccliff2@hotmail.com
Journal of veterinary internal medicine / American College of Veterinary Internal Medicine (United States) Mar-Apr 2001, 15 (2) p131-5, ISSN 0891-6640 Journal Code: 8708660
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Vascular endothelial growth factor (*VEGF*) is a *dimeric* glycosylated polypeptide growth factor with potent angiogenic, mitogenic, and vascular permeability-enhancing properties specific for endothelial cells. In humans, *VEGF* seems to play a major role in tumor growth, and plasma concentrations correlate with tumor burden, response to therapy, and disease progression. This study compared plasma *VEGF* concentrations in healthy client-owned dogs (n = 17) to dogs with hemangiosarcoma (HSA; n 16). Dogs with HSA were significantly more likely to have detectable concentrations of plasma *VEGF* (13/17) compared to healthy dogs (1/17; P < .001). The median plasma *VEGF* concentration for dogs with HSA was 17.2 pg/mL (range, < 1.0-66.7 pg/mL). Plasma *VEGF* concentrations in dogs with HSA did not correlate with stage of disease or tumor burden, but 1 dog had undetectable *VEGF* during chemotherapy that subsequently increased with disease progression.

10/3,AB/10 (Item 10 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

11125182 21134510 PMID: 11238106
Exogenous clustered neuropilin 1 enhances vasculogenesis and angiogenesis.
Yamada Y; Takakura N; Yasue H; Ogawa H; Fujisawa H; Suda T Department of Cell Differentiation, Institute of Molecular Embryology and Genetics, University School of Medicine, Kumamoto, Japan.
Blood (United States) Mar 15 2001, 97 (6) p1671-8, ISSN 0006-4971 Journal Code: 7603509
Document type: Journal Article
Languages: ENGLISH

Main Citation Owner: NLM
Record type: Completed
Neuropilin 1 (NP-1) is a receptor for vascular endothelial growth factor (*VEGF*) 165 (VEGF165) and acts as a coreceptor that enhances VEGF165 function through tyrosine kinase *VEGF* receptor 2 (VEGFR-2). Transgenic overexpression of np-1 results in an excess of capillaries and blood vessels and a malformed heart. Thus, NP-1 may have a key role in vascular development. However, how NP-1 regulates vascular development is not well understood. This study demonstrates how NP-1 can regulate vasculogenesis and angiogenesis in vitro and in vivo. In homozygous np-1 mutant (np-1(-/-)) murine embryos, vascular sprouting was impaired in the central nervous system and pericardium. Para-aortic splanchnopleural mesoderm (P-Sp) explants from np-1(-/-) mice also had vascular defects in vitro. A monomer of soluble NP-1 (NP-1 tagged with Flag epitope) inhibited vascular development in cultured wild-type P-Sp explants by sequestering VEGF165. In contrast, a *dimer* of soluble NP-1 (NP-1 fused with the Fc part of human IgG) enhanced vascular development in cultured wild-type P-Sp explants. Moreover, the NP-1-Fc rescued the defective vascular development in cultured np-1(-/-) P-Sp explants. A low dose of *VEGF* alone did not promote phosphorylation of VEGFR-2 on endothelial cells from np-1(-/-) embryos, but simultaneous addition of a low dose of *VEGF* and NP-1-Fc phosphorylated VEGFR-2 significantly. Moreover, NP-1-Fc rescued the defective vascularity of np-1(-/-) embryos in vivo. These results suggest that a *dimer* form of soluble NP-1 delivers VEGF165 to VEGFR-2-positive endothelial cells and promotes angiogenesis.

10/3,AB/11 (Item 11 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10926531 20490260 PMID: 11035661
Serum levels of vascular endothelial growth factor dependent on the stage progression of lung cancer.
Matsuyama W; Hashiguchi T; Mizoguchi A; Iwami F; Kawabata M; Arimura K; Osame M
Third Department of Internal Medicine, Kagoshima University School of Medicine, Kagoshima City, Japan.
Chest (UNITED STATES) Oct 2000, 118 (4) p948-51, ISSN 0012-3692 Journal Code: 0231335
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
STUDY OBJECTIVE: In lung cancer, vascular endothelial growth factor (*VEGF*) is an important cytokine and is correlated with tumor vessel density, malignant pleural effusions, and coagulation-fibrinolysis factors in vitro. We investigated the correlation between serum *VEGF* level and stage progression in lung cancer to study the predicted value of *VEGF* level. We also studied whether coagulation-fibrinolysis factors and PaO(2) levels, which are also important factors for the prediction of the clinical course, are correlated with *VEGF*. METHODS: Forty-nine patients with lung cancer were investigated prospectively. *VEGF* levels of sera and malignant effusions, and plasma concentrations of coagulation-fibrinolysis factors were measured by enzyme-linked immunosorbent assay. We measured PaO(2) levels in all patients at rest. RESULTS: Serum levels of *VEGF* were increased significantly according to stage progression. Additionally, plasma concentrations of D *dimer*, thrombin-antithrombin complex (TAT), and tissue plasminogen activator/plasminogen activator inhibitor type I complex were elevated significantly according to stage progression. The serum *VEGF* level had a significant positive correlation with the TAT and D *dimer* levels. Serum *VEGF* levels had a significant negative correlation with PaO(2) levels. The incidence of cerebral vascular disorder was significantly higher in the patients with systemic hypoxemia than in those without ($p<0.05$). Mean *VEGF* levels in malignant effusions in eight patients (five with pleural effusions, two with pericardial effusions, and one with both) were extremely high, especially in pericardial effusions ([mean +/- SD] pleural effusions, 531.9+/-285.4 pg/mL; pericardial effusion, 3,071.6+/-81.3 pg/mL). CONCLUSION: We predict that in lung cancer, *VEGF* production and the abnormality of the coagulation-fibrinolysis system differ depending on the stage of progression of disease. Serum *VEGF* levels would be affected by PaO(2) levels in lung cancer.

10/3,AB/12 (Item 12 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10818680 20375645 PMID: 10919072
Recombinant production of PIGF-1 and its activity in animal models. Maglione D; Battisti M; Tucci M
Farmaco (Societa chimica italiana : 1989) (ITALY) Mar 2000, 55 (3) p165-7, ISSN 0014-827X Journal Code: 8912641
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
In this paper we review current knowledge on placenta growth factor (PIGF) and summarise our data on its recombinant production in bacteria and its activity. PIGF and vascular endothelial growth factor (*VEGF*) are both angiogenic factors belonging to the platelet-derived growth factor (PDGF) family. PIGF is a *dimeric* glycoprotein which shares a number of biochemical and functional features with *VEGF*. The

aminoacidic similarity between the two factors is high (about 50%) in the PDGF-like domain. By alternative splicing of the PIGF mRNA, three forms of PIGF protein are generated which are named PIGF-1, PIGF-2 and PIGF-3. We have focused our attention on form 1 of human PIGF (PIGF-1). A large quantity of active recombinant PIGF-1 has been obtained using a bacterial expression system. By optimising the fermentation and purification it was possible to produce about 140 mg/l of culture of active PIGF-1, which is potentially suitable for a pharmaceutical use. The angiogenic activity of two different batches of bacteria-derived PIGF-1 obtained in our laboratory was demonstrated in chick chorionallantoic membrane assays. Finally, in preliminary studies we have shown that bacteria-derived PIGF-1 has a protective effect against myocardial lesions induced by isoprenaline in rat and rabbit.

10/3,AB/13 (Item 13 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10794272 20341076 PMID: 10880433
Structure of the active core of human stem cell factor and analysis of binding to its receptor kit.
Jiang X; Gurel O; Mendiaz E A; Stearns G W; Clogston C L; Lu H S; Osslund T D; Syed R S; Langley K E; Hendrickson W A
Department of Biochemistry and Molecular Biophysics and Howard Hughes Medical Institute, Columbia University, New York, NY 10032, USA. EMBO journal (ENGLAND) Jul 3 2000, 19 (13) p3192-203, ISSN 0261-4189 Journal Code: 8208664
Contract/Grant No.: GM-34102; GM; NIGMS
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Stem cell factor (SCF) is an early-acting hematopoietic cytokine that elicits multiple biological effects. SCF is *dimeric* and occurs in soluble and membrane-bound forms. It transduces signals by ligand-mediated dimerization of its receptor, Kit, which is a receptor tyrosine kinase related to the receptors for platelet-derived growth factor (PDGF), macrophage colony-stimulating factor, Flt-3 ligand and vascular endothelial growth factor (*VEGF*). All of these have extracellular ligand-binding portions composed of immunoglobulin-like repeats. We have determined the crystal structure of selenomethionyl soluble human SCF at 2.2 A resolution by multiwavelength anomalous diffraction phasing. SCF has the characteristic helical cytokine topology, but the structure is unique apart from core portions. The SCF *dimer* has a symmetric 'head-to-head' association. Using various prior observations, we have located potential Kit-binding sites on the SCF *dimer*. A superimposition of this *dimer* onto *VEGF* in its complex with the receptor Flt-1 places the binding sites on SCF in positions of topographical and electrostatic complementarity with the Kit counterparts of Flt-1, and a similar model can be made for the complex of PDGF with its receptor.

10/3,AB/14 (Item 14 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10787817 20345531 PMID: 10886548
Hypoxia and interleukin-1beta stimulate vascular endothelial growth factor production in human proximal tubular cells.
El Awad B; Kreft B; Wolber E M; Hellwig-Burgel T; Metzen E; Fandrey J; Jelkmann W
Institute of Physiology and Department of Internal Medicine I, Medical University of Lubeck, Germany.
Kidney international (UNITED STATES) Jul 2000, 58 (1) p43-50, ISSN 0085-2538 Journal Code: 0323470
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
BACKGROUND: Vascular endothelial growth factor (*VEGF*) promotes angiogenesis and inflammatory reactions. *VEGF* mRNA is detectable in the proximal tubules of inflamed kidneys but not in normals. In other organs *VEGF* gene expression is induced by hypoxia and cytokines such as interleukin 1 (IL-1). To identify the cellular mechanisms in control of tubular *VEGF* production, we studied effects of hypoxia and IL-1beta in *VEGF* mRNA levels, *VEGF* secretion, and activity of the hypoxia-inducible *dimeric* transcription factor 1 (HIF-1alpha/beta) in human proximal tubular epithelial cells (PTECs) in primary culture. METHODS: PTECs were grown in monolayers from human kidneys. Hypoxia was induced by incubation at 3% O2. *VEGF* mRNA was quantitated by competitive polymerase chain reaction following reverse transcription. *VEGF* was measured by enzyme-linked immunoassay. HIF-1alpha was demonstrated by Western blot analysis and HIF-1 DNA binding by gel shift assay. RESULTS: Significant amounts of *VEGF* mRNA and *VEGF* protein were measured in PTEC extracts and culture media, respectively. Stimulation of *VEGF* synthesis at low O2 tension and following IL-1beta treatment was detectable at the protein level only. Nuclear HIF-1alpha protein levels and HIF-1 binding to DNA were also increased under these conditions. CONCLUSIONS: PTECs in culture produce *VEGF*. One mechanism of induction appears to be increased DNA binding of HIF-1 to hypoxia-responsive elements in

the *VEGF* gene promoter. In inflammatory diseases of the kidney, tubular cell-derived *VEGF* may contribute to microvascular leakage and monocyte extravasation.

10/3,AB/15 (Item 15 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10487797 20011413 PMID: 10542248
Biosynthesis of vascular endothelial growth factor-D involves proteolytic processing which generates non-covalent homodimers.
Stacker S A; Stenvers K; Caesar C; Vitali A; Domagala T; Nice E; Roufail S; Simpson R J; Moritz R; Karpanen T; Alitalo K; Achen M G Ludwig Institute for Cancer Research, Royal Melbourne Hospital, Parkville, Victoria 3050, Australia. steven.stacker@ludwig.edu.au Journal of biological chemistry (UNITED STATES) Nov 5 1999, 274 (45) p32127-36, ISSN 0021-9258 Journal Code: 2985121R
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Vascular endothelial growth factor-D (*VEGF*-D) binds and activates the endothelial cell tyrosine kinase receptors *VEGF* receptor-2 (VEGFR-2) and *VEGF* receptor-3 (VEGFR-3), is mitogenic for endothelial cells, and shares structural homology and receptor specificity with *VEGF*-C. The primary translation product of *VEGF*-D has long N- and C-terminal polypeptide extensions in addition to a central *VEGF* homology domain (VHD). The VHD of *VEGF*-D is sufficient to bind and activate VEGFR-2 and VEGFR-3. Here we report that *VEGF* -D is proteolytically processed to release the VHD. Studies in 293EBNA cells demonstrated that *VEGF*-D undergoes N- and C-terminal cleavage events to produce numerous secreted polypeptides including a fully processed form of M(r) approximately 21,000 consisting only of the VHD, which is predominantly a non-covalent *dimer*. Biosensor analysis demonstrated that the VHD has approximately 290- and approximately 40-fold greater affinity for VEGFR-2 and VEGFR-3, respectively, compared with unprocessed *VEGF* -D. In situ hybridization demonstrated that embryonic lung is a major site of expression of the *VEGF*-D gene. Processed forms of *VEGF*-D were detected in embryonic lung indicating that *VEGF*-D is proteolytically processed in vivo.

10/3,AB/16 (Item 16 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10452748 99458710 PMID: 10529242
Kinetics and thermodynamics of *dimer* formation and dissociation for a recombinant humanized monoclonal antibody to vascular endothelial growth factor.
Moore J M; Patapoff T W; Cromwell M E
Department of Pharmaceutical R&D, Genentech, Inc., South San Francisco, California 94080, USA.
Biochemistry (UNITED STATES) Oct 19 1999, 38 (42) p13960-7, ISSN 0006-2960 Journal Code: 0370623
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The recombinant humanized antibody (rhuMAb) *VEGF* has a high affinity for vascular endothelial growth factor and is currently being evaluated in clinical trials as a cancer therapeutic. Under acidic pH and low ionic strength conditions, the antibody was predominantly present as monomer. Under physiological conditions, the appearance of significant amounts of a noncovalent, reversible *dimer* were observed by size-exclusion chromatography. The kinetics and thermodynamics of the reversible self-association for rhuMAb *VEGF* monomer were investigated as a function of pH, temperature, and ionic strength by size-exclusion chromatography using the concentration jump method. The rate constant for *dimer* formation ranged 23-112 M(-)(1) min(-)(1) under the conditions studied, values that are significantly lower than those reported in the literature for other proteins that self-associate. The rate constant for dissociation ranged 0.0039-0.021 min(-)(1). Gibbs' free energies, enthalpies, entropies, and activation energies were determined and revealed that *dimer* formation is optimal at pH 7.5-8.0, which may be reflective of charge shielding occurring near the pI of the protein. There was a negative change in entropy for dissociation (values from -18.1 to -12.8 cal/mol K). In the presence of D(2)O or 1 M NaCl, dimerization was enhanced. The results of the kinetic and thermodynamic analysis of this study indicate that rhuMAb *VEGF* dimerization occurs primarily through hydrophobic interactions.

10/3,AB/17 (Item 17 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10307122 99290233 PMID: 10363565
Characterization of a new potent, in vivo neutralizing monoclonal antibody to human vascular endothelial growth factor.
Schlaeppi J M; Siemeister G; Weindel K; Schnell C; Wood J Novartis Pharmaceuticals, Core Technology Department, Novartis Limited, Basel, Switzerland. jean-marc.schlaeppi@pharma.novartis.com Journal of cancer research and clinical oncology (GERMANY) 1999,

125 (6) p336-42, ISSN 0171-5216 Journal Code: 7902060
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Vascular endothelial growth factor (*VEGF*) is an important mediator of tumor-induced angiogenesis and represents a potential target for anticancer therapy. Therefore, we prepared a panel of monoclonal antibodies (mAb) against both the VEGF121 and VEGF165 isoforms. Three of them completely neutralized the mitogenic stimulation by *VEGF* of human umbilical vein endothelial cells at mAb concentrations below 0.1 microg/ml. The most potent one, with a dissociation constant (Kd) of 8 pM, inhibited, in a dose-dependent manner, *VEGF*-induced angiogenesis in a growth factor implant model in mice. A complete inhibition of the angiogenic response was obtained by daily intraperitoneal injections of 10 microg mAb/mouse. Angiogenesis induced by basic fibroblast growth factor was not inhibited by the mAb. Epitope mapping of the mAb, performed by competitive enzyme-linked immunosorbent assay and Western blot analysis, showed that it did not bind to the reduced and denatured monomer of *VEGF* . Substitutions of three residues (Q87R, G88K, Q89K), located on the major surface loop beta5 to beta6 of *VEGF*, resulted in the complete loss of binding (more than 400-fold reduction). The results suggest that the mAb binds primarily to a conformation-dependent epitope on the *VEGF* *dimeric* form, encompassing one of the loop regions involved in KDR receptor binding. The mAb with its strong neutralizing properties represents a useful agent for effective blocking of *VEGF*-mediated tumor neovascularization.

10/3,AB/18 (Item 18 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10278959 99262355 PMID: 10328919
Defective vascularization of HIF-1alpha-null embryos is not associated with *VEGF* deficiency but with mesenchymal cell death. Kotch L E; Iyer N V; Laughner E; Semenza G L
Institute of Genetic MedicineDepartment of PediatricsDepartment of Medicine, Johns Hopkins University School of Medicine, Baltimore, Maryland, 21287-3914, USA.
Developmental biology (UNITED STATES) May 15 1999, 209 (2) p254-67, ISSN 0012-1606 Journal Code: 0372762
Contract/Grant No.: RO1-HL55338; HL; NHLBI
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Hypoxia-inducible factor 1 (HIF-1) is a *dimeric* transcription factor composed of HIF-1alpha and HIF-1beta subunits that plays an essential role in mammalian O2 homeostasis. In Hif1a-/- knockout mice, complete deficiency of HIF-1alpha resulted in cardiac and vascular malformations and embryonic lethality at E10.5. Between E8. 75 and E9.25 striking vascular regression and abnormal remodeling occurred in the cephalic region concomitant with marked mesenchymal cell death. Similar vascular defects were observed in HIF-1alpha- and *VEGF*-deficient embryos and *VEGF* mRNA expression was not induced by hypoxia in Hif1a-/- embryonic stem cells. Surprisingly, Hif1a-/- embryos demonstrated increased *VEGF* mRNA expression compared to wild-type embryos. In tissue culture cells, *VEGF* mRNA expression was induced by glucose deprivation independent of HIF-1alpha, providing a mechanism for increased *VEGF* mRNA expression in Hif1a-/- embryos, in which absence of adequate tissue perfusion resulted in both O2 and glucose deprivation. Rather than being associated with *VEGF* deficiency, the vascular defects in Hif1a-/- embryos were spatially correlated with cell death, the onset of which preceded vascular regression. Copyright 1999 Academic Press.

10/3,AB/19 (Item 19 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10129193 99120976 PMID: 9920854
Antineoplastic urinary protein inhibits Kaposi's sarcoma and angiogenesis in vitro and in vivo.
Masood R; McGarvey M E; Zheng T; Cai J; Arora N; Smith D L; Sloane N; Gill P S
University of Southern California School of Medicine, Department of Internal Medicine and Pathology, Los Angeles, CA, USA.
Blood (UNITED STATES) Feb 1 1999, 93 (3) p1038-44, ISSN 0006-4971 Journal Code: 7603509
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Kaposi's sarcoma (KS) is the most common tumor in human immunodeficiency virus infection and acquired immune deficiency syndrome. Recent clinical trials with human chorionic gonadotropin (hCG) prepared from early pregnancy urine have shown encouraging results in the resolution of KS lesions. A urinary protein with antitumor activity, ANUP (antineoplastic urinary protein), a *dimer* of 32 kD, has previously been shown to inhibit the growth of various tumor cell lines in vivo. It was thus studied for its activity in KS cell lines in vitro and in vivo to determine whether it could be a source of the anti-KS

activity observed in hCG preparations. ANUP is a strong growth inhibitor for KS cell lines, but has little or no effect on fibroblast, aortic smooth muscle, T- and B-lymphocyte, and monocyte cell lines. ANUP also inhibited the proliferation of endothelial cell lines, suggesting that the in vitro effects were endothelial cell lineage-specific. However, ANUP antibodies did not block the inhibitory effect of certain commercial preparations of hCG, previously shown to be active in KS. Thus, the active protein in these commercial preparations of hCG may be distinct from ANUP. The antitumor activity of ANUP was further confirmed in a chicken allantoic membrane (CAM) assay in which vascular endothelial growth factor (*VEGF*) and beta fibroblast growth factor (bFGF)-induced angiogenesis was inhibited by ANUP in a dose-dependent manner. In vivo activity of ANUP was demonstrated in the murine model of KS, where ANUP inhibited tumor growth. ANUP is thus a potential candidate for development in the treatment of KS and other diseases in which angiogenesis plays an important role.

10/3,AB/20 (Item 20 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10071049 99060122 PMID: 9843450
Characterization and kinetic mechanism of catalytic domain of human vascular endothelial growth factor receptor-2 tyrosine kinase (VEGFR2 TK), a key enzyme in angiogenesis.
Parast C V; Mroczkowski B; Pinko C; Misialek S; Khambatta G; Appelt K Agouron Pharmaceuticals, Incorporated, San Diego, California 92121, USA. parast@agouron.com
Biochemistry (UNITED STATES) Nov 24 1998, 37 (47) p16788-801, ISSN 0006-2960 Journal Code: 0370623
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Vascular endothelial growth factor (*VEGF*) is a *dimeric* protein which induces formation of new blood vessels (angiogenesis) through binding to *VEGF*-receptor-2 tyrosine kinase (VEGFR2 TK) or KDR (kinase insert domain-containing receptor) on the surface of endothelial cells. Angiogenesis has been shown to be essential for malignancy of tumors; therefore, VEGFR2 TK is a potential therapeutic target for the treatment of cancer. Sequence homology studies indicate that VEGFR2 TK contains three domains: extracellular (ligand-binding domain), transmembrane, and intracellular (catalytic domain). In this work, the catalytic domain of VEGFR2 TK was cloned and expressed in a soluble active form using a baculovirus expression system. In the absence of ligand, the enzyme is shown to catalyze its autophosphorylation in a time-dependent and enzyme-concentration-dependent manner, consistent with a trans mechanism for this reaction. Mass spectrometry analysis revealed incorporation of 5.5 +/- 0.5 mol of phosphate/mole of enzyme (monomer). In addition, the enzyme was shown to catalyze phosphorylation of a synthetic peptide, poly(E4Y). Using poly(E4Y) as substrate, the kinetic constants of both native and phosphorylated enzyme were determined. Enzyme phosphorylation increased catalytic efficiency of the enzyme by at least an order of magnitude. Furthermore, the enzyme was shown to catalyze the reverse reaction using phospho-poly(E4Y) as substrate. Cd2+ was found to be an inhibitor of the enzyme. Kinetic studies revealed that inhibition by Cd2+ was competitive with respect to Mg2+ and noncompetitive with respect to MgATP. These results indicate that Cd2+ competes for a second metal-binding site. Therefore, the reaction catalyzed by this enzyme was treated as a terreactant system. The kinetic mechanism of VEGFR2 TK was elucidated through the use of steady-state kinetic studies. According to these studies, the enzyme binds Mg2+ and MgATP in a random fashion followed by ordered addition of the peptide substrate. The release of product is also ordered, with MgADP being released last. The order of substrate binding was confirmed by using AMP-PCP, a dead-end inhibitor.

10/3,AB/21 (Item 21 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10042324 99030400 PMID: 9813035
A novel type of vascular endothelial growth factor, *VEGF*-E (NZ-7 *VEGF*), preferentially utilizes KDR/Flk-1 receptor and carries a potent mitotic activity without heparin-binding domain.
Ogawa S; Oku A; Sawano A; Yamaguchi S; Yazaki Y; Shibuya M Department of Genetics, Institute of Medical Science, University of Tokyo, Minato-ku, Tokyo 108-8639 Japan.
Journal of biological chemistry (UNITED STATES) Nov 20 1998, 273 (47) p31273-82, ISSN 0021-9258 Journal Code: 2985121R
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Vascular endothelial growth factor (*VEGF*) mediates endothelial cell proliferation, angiogenesis, and vascular permeability via the endothelial cell receptors, KDR/Flk-1 and Flt-1. Recently, a gene encoding a polypeptide with about 25% amino acid identity to

mammalian *VEGF* was identified in the genome of Orf virus (OV), a parapoxvirus that affects sheep and goats and occasionally, humans, to generate lesions with angiogenesis. In this study, we examined the biological activities and receptor of OV-derived NZ-7 *VEGF* (*VEGF*-E). *VEGF*-E was found to be a *dimer* of about 20 kDa with no basic domain nor affinity for heparin column, similar to VEGF121 subtype. VEGF121 has 10-100-fold less endothelial cell mitotic activity than VEGF165 due to lack of a heparin-binding basic region. Interestingly, however, *VEGF*-E showed almost equal levels of mitotic activity on primary endothelial cells and vascular permeability activity as VEGF165. Furthermore, *VEGF*-E bound KDR/Flk-1 (VEGFR-2) and induced its autophosphorylation to almost the same extent as VEGF165, but did not bind Flt-1 (VEGFR-1) nor induce autophosphorylation of Flt-1. These results indicate that *VEGF*-E is a novel type of endothelial growth factor, utilizing only one of the *VEGF* receptors, and carrying a potent mitogenic activity without affinity to heparin.

10/3,AB/22 (Item 22 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09992709 98428671 PMID: 9753694
VEGF and the Fab fragment of a humanized neutralizing antibody: crystal structure of the complex at 2.4 A resolution and mutational analysis of the interface.
Muller Y A; Chen Y; Christinger H W; Li B; Cunningham B C; Lowman H B; de Vos A M
Department of Protein Engineering Genentech, Inc. 1 DNA Way, South San Francisco, CA 94080, USA.
Structure (London, England) (ENGLAND) Sep 15 1998, 6 (9) p1153-67, ISSN 0969-2126 Journal Code: 9418985
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
BACKGROUND: Vascular endothelial growth factor (*VEGF*) is a highly specific angiogenic growth factor; anti-angiogenic treatment through inhibition of receptor activation by *VEGF* might have important therapeutic applications in diseases such as diabetic retinopathy and cancer. A neutralizing anti-*VEGF* antibody shown to suppress tumor growth in an in vivo murine model has been used as the basis for production of a humanized version. RESULTS: We present the crystal structure of the complex between *VEGF* and the Fab fragment of this humanized antibody, as well as a comprehensive alanine-scanning analysis of the contact residues on both sides of the interface. Although the *VEGF* residues critical for antibody binding are distinct from those important for high-affinity receptor binding, they occupy a common region on *VEGF*, demonstrating that the neutralizing effect of antibody binding results from steric blocking of *VEGF*-receptor interactions. Of the residues buried in the *VEGF* -Fab interface, only a small number are critical for high-affinity binding; the essential *VEGF* residues interact with those of the Fab fragment, generating a remarkable functional complementarity at the interface. CONCLUSIONS: Our findings suggest that the character of antigen-antibody interfaces is similar to that of other protein-protein interfaces, such as ligand-receptor interactions; in the case of *VEGF*, the principal difference is that the residues essential for binding to the Fab fragment are concentrated in one continuous segment of polypeptide chain, whereas those essential for binding to the receptor are distributed over four different segments and span across the *dimer* interface.

10/3,AB/23 (Item 23 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09803736 98225203 PMID: 9556609
Requirements for binding and signaling of the kinase domain receptor for vascular endothelial growth factor.
Fuh G; Li B; Crowley C; Cunningham B; Wells J A
Department of Protein Engineering, Genentech, Inc., South San Francisco, California 94080, USA.
Journal of biological chemistry (UNITED STATES) May 1 1998, 273 (18) p11197-204, ISSN 0021-9258 Journal Code: 2985121R
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Vascular endothelial growth factor (*VEGF*) is a *dimeric* hormone that controls much of vascular development through binding and activation of its kinase domain receptor (KDR). We produced analogs of *VEGF* that show it has two receptor-binding sites which are located near the poles of the *dimer* and straddle the interface between subunits. Deletion experiments in KDR indicate that of the seven IgG-like domains in the extracellular domain, only domains 2-3 are needed for tight binding of *VEGF*. Monomeric forms of the extracellular domain of KDR bind approximately 100 times weaker than *dimeric* forms showing a strong avidity component for binding of *VEGF* to predimerized forms of the receptor. Based upon these structure-function studies and a mechanism in which receptor dimerization is critical for signaling, we constructed a receptor antagonist in the form of a heterodimer of *VEGF* that contained one functional and one non-functional site. These studies establish a

functional foundation for the design of *VEGF* analogs, mimics, and antagonists.

10/3,AB/24 (Item 24 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09803724 98225191 PMID: 9556597
The alpha-helical domain near the amino terminus is essential for dimerization of vascular endothelial growth factor.
Siemeister G; Marme D; Martiny-Baron G
Institute of Molecular Medicine, Tumor Biology Center, D-79106 Freiburg, Germany.
Journal of biological chemistry (UNITED STATES) May 1 1998, 273 (18) p11115-20, ISSN 0021-9258 Journal Code: 2985121R
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Vascular endothelial growth factor (*VEGF*) is an endothelial cell-specific mitogen and a key mediator of aberrant endothelial cell proliferation and vascular permeability in a variety of human pathological situations such as tumor angiogenesis, diabetic retinopathy, or psoriasis. By amino-terminal deletion analysis and by site-directed mutagenesis we have identified a new domain within the amino-terminal alpha-helix that is essential for dimerization of *VEGF*. VEGF121 variants containing amino acids 8 to 121 or 14 to 121, respectively, either expressed in Escherichia coli and refolded in vitro, or expressed in Chinese hamster ovary cells, were in a *dimeric* conformation and showed full binding activity to *VEGF* receptors and stimulation of endothelial cell proliferation as compared with wild-type *VEGF*. In contrast, a VEGF121 variant covering amino acids 18 to 121, as well as a variant in which the hydrophobic amino acids Val14, Val15, Phe17, and Met18 within the amphipathic alpha-helix near the amino terminus were replaced by serine, failed to form biological active *VEGF* dimers. From these data we conclude that a domain between amino acids His12 and Asp19 within the amino-terminal alpha-helix is essential for formation of *VEGF* dimers, and we propose hydrophobic interactions between *VEGF* monomers to stabilize or favor dimerization.

10/3,AB/25 (Item 25 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09776999 98208591 PMID: 9539788
An antagonistic vascular endothelial growth factor (*VEGF*) variant inhibits *VEGF* -stimulated receptor autophosphorylation and proliferation of human endothelial cells.
Siemeister G; Schirner M; Reusch P; Barleon B; Marme D; Martiny-Baron G Institute of Molecular Medicine, Tumor Biology Center, Freiburg, Germany. Proceedings of the National Academy of Sciences of the United States of America (UNITED STATES) Apr 14 1998, 95 (8) p4625-9, ISSN 0027-8424 Journal Code: 7505876
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Vascular endothelial growth factor (*VEGF*) is a potent mitogen with a unique specificity for endothelial cells and a key mediator of aberrant endothelial cell proliferation and vascular permeability in a variety of human pathological situations, such as tumor angiogenesis, diabetic retinopathy, rheumatoid arthritis, or psoriasis. *VEGF* is a symmetric homodimeric molecule with two receptor binding interfaces lying on each pole of the molecule. Herein we report on the construction and recombinant expression of an asymmetric heterodimeric *VEGF* variant with an intact receptor binding interface at one pole and a mutant receptor binding interface at the second pole of the *dimer*. This *VEGF* variant binds to *VEGF* receptors but fails to induce receptor activation. In competition experiments, the heterodimeric *VEGF* variant antagonizes *VEGF* -stimulated receptor autophosphorylation and proliferation of endothelial cells. A 15-fold excess of the heterodimer was sufficient to inhibit *VEGF*-stimulated endothelial cell proliferation by 50%, and a 100-fold excess resulted in an almost complete inhibition. By using a rational approach that is based on the structure of *VEGF*, we have shown the feasibility to construct a *VEGF* variant that acts as an *VEGF* antagonist.

10/3,AB/26 (Item 26 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09663363 98086121 PMID: 9426152
Pattern and localisation of expression of vascular endothelial growth factor and its receptor flt-1 in the ovine pituitary gland: expression is independent of hypothalamic control.
Jabbour H N; Boddy S C; Lincoln G A
MRC Reproductive Biology Unit, Edinburgh, UK. h.jabbour@ed-rbu.mrc.ac.uk Molecular and cellular endocrinology (IRELAND) Nov 15 1997, 134 (2) p91-100, ISSN 0303-7207 Journal Code: 7500844
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The pituitary gland, a highly vascularised endocrine

organ, contains permeable fenestrated endothelium that allows direct access of endocrine cells to the hemal milieu. Vascular endothelial growth factor (*VEGF*) has a mitogenic effect on endothelial cells and renders the endothelium more permeable. The following study investigated the expression of *VEGF* and its receptor flt-1 mRNA and protein in the pituitary gland of sheep. *VEGF* expression was localised, by in situ hybridisation and immunocytochemistry, mainly to the pars tuberalis/zona tuberalis (PT/ZT) region of the gland. No hybridisation signal was observed in the pars intermedia or pars nervosa. Reverse transcriptase-polymerase chain reaction (RT-PCR) Southern blotting confirmed the predominant expression of *VEGF* in the PT/ZT compared with the pars distalis (PD). Western blot analysis with the *VEGF* antibody revealed major (48 kDa) and minor (24 kDa) bands representing the monomer and *dimer* forms of *VEGF* and also confirmed the differential expression of *VEGF* in the PT/ZT compared with the PD. Double immunocytochemistry with *VEGF* and prolactin or luteinising hormone-beta (LH-beta) antibodies demonstrated that the *VEGF* -secreting cells are not lactotrophs or gonadotrophs. However, co-localisation of *VEGF* with S-100 was observed in a proportion of cells suggesting that some *VEGF* secreting cells are follicular stellate. Immunocytochemistry with a flt-1 antibody confirmed the expression of this high affinity receptor for *VEGF* in endothelial cells across the pituitary gland. Immunocytochemistry with the *VEGF* antibody using pituitary glands from intact and hypothalamo-pituitary disconnected sheep demonstrated comparable expression patterns suggesting that the regulation of blood flow and vascular permeability in the pituitary gland is under local regulation and is independent of hypothalamic input.

10/3,AB/27 (Item 27 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09624967 98054011 PMID: 9393862
Crystal structure at 1.7 A resolution of *VEGF* in complex with domain 2 of the Flt-1 receptor.
Wiesmann C; Fuh G; Christinger H W; Eigenbrot C; Wells J A; de Vos A M Genentech, Inc., Department of Protein Engineering, South San Francisco, California 94080, USA.
Cell (UNITED STATES) Nov 28 1997, 91 (5) p695-704, ISSN 0092-8674 Journal Code: 0413066
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Vascular endothelial growth factor (*VEGF*) is a homodimeric hormone that induces proliferation of endothelial cells through binding to the kinase domain receptor and the Fms-like tyrosine kinase receptor (Flt-1), the extracellular portions of which consist of seven immunoglobulin domains. We show that the second and third domains of Flt-1 are necessary and sufficient for binding *VEGF* with near-native affinity, and that domain 2 alone binds only 60-fold less tightly than wild-type. The crystal structure of the complex between *VEGF* and the second domain of Flt-1 shows domain 2 in a predominantly hydrophobic interaction with the "poles" of the *VEGF* *dimer*. Based on this structure and on mutational data, we present a model of *VEGF* bound to the first four domains of Flt-1.

10/3,AB/28 (Item 28 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09618208 98036675 PMID: 9369935
Characterization of the extracellular domain in vascular endothelial growth factor receptor-1 (Flt-1 tyrosine kinase).
Tanaka K; Yamaguchi S; Sawano A; Shibuya M
Department of Genetics, University of Tokyo.
Japanese journal of cancer research : Gann (JAPAN) Sep 1997, 88 (9) p867-76, ISSN 0910-5050 Journal Code: 8509412
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Flt-1 tyrosine kinase, vascular endothelial growth factor (*VEGF*) receptor-1, binds *VEGF* and a new *VEGF*-related ligand, placenta growth factor, but KDR/Flk-1 (*VEGF* receptor-2) binds only *VEGF*. To characterize the functional regions in the Flt-1 extracellular domain such as the ligand binding region and the *dimer* formation of the receptor, we constructed a series of mutants of the Flt-1 extracellular domain as soluble forms in a baculovirus system. We found that a region carrying the N-terminal 1st to 3rd immunoglobulin (Ig)-like domains of Flt-1 binds both ligands with high affinity. However, for *dimer* formation of soluble Flt-1, a region further downstream in the Flt-1 extracellular domain was required. Mutant Flt-1 receptors expressed in COS cells confirmed the requirement of the 4th to 7th Ig region for the activation of Flt-1 tyrosine kinase. Soluble Flt-1 carrying the N-terminal 1st to 3rd Ig region suppressed *VEGF* -dependent endothelial proliferation in vitro to the same level as the larger forms of soluble Flt-1, suggesting that the binding of one soluble Flt-1 molecule to one subunit of the *VEGF* homodimer may be sufficient to block the *VEGF* activity.

10/3,AB/29 (Item 29 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09613932 98048230 PMID: 9386989
Characterization of a soluble vascular endothelial growth factor receptor-immunoglobulin chimera.
Kaplan J B; Sridharan L; Zaccardi J A; Dougher-Vermazen M; Terman B I Oncology Section, Wyeth-Ayerst Research, Pearl River, New York 10965, USA.
Growth factors (Chur, Switzerland) (SWITZERLAND) 1997, 14 (4) p243-56, ISSN 0897-7194 Journal Code: 9000468
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
To investigate the interaction between vascular endothelial growth factor (*VEGF*) and its receptor, we have constructed a chimeric protein consisting of the extracellular ligand-binding domain of the human *VEGF* receptor subtype KDR fused to a human IgG1 Fc domain (KDR-Fc). KDR-Fc was expressed in human 293 kidney epithelial cells as a 300-kDa secreted, *dimeric* glycoprotein that bound 125I-VEGF165 with high affinity (Kd = 150 pM). Unlike the full length cellular receptor, KDR-Fc did not require heparin for 125I-VEGF165 binding, although heparin did stimulate 125I-VEGF165 binding approximately 50 to 100%. Similar results were observed for KDR-Fc expressed in yeast cells. Since yeast do not synthesize heparan sulfate proteoglycans, we conclude that cellular heparan sulfates do not account for the lack of a heparin requirement for 125I-VEGF165 binding to KDR-Fc. The polycationic protein protamine, which inhibits (IC50 = 1 microgram/ml) 125I-VEGF165 binding to bovine aortic endothelial cells and other KDR-expressing cells by blocking heparin interactions, had no effect on the heparin independent component of 125I-VEGF165 binding to KDR-Fc. Protamine does inhibit (IC50 = 1 microgram/ml) the heparin dependent component of 125I-VEGF165 binding to KDR-Fc. KDR-Fc bound VEGF121 with the same affinity as VEGF165. Heparin had no effect on 125I-VEGF121 binding to KDR-Fc, indicating that heparin interaction with the 44 amino acids contained in VEGF165 but not VEGF121 allow for maximal VEGF165 binding. Deletion analysis of KDR-Fc demonstrated that the determinants required for high affinity *VEGF* binding are located in the three aminoterminal Ig-domains of the protein. Heparin had no effect on 125I-VEGF165 binding to the three Ig-domain receptor, suggesting that there are heparin binding determinants located in KDR Ig-domains 4 to 7.

10/3,AB/30 (Item 30 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09138847 97030254 PMID: 8876195
Identification of a c-fos-induced gene that is related to the platelet-derived growth factor/vascular endothelial growth factor family. Orlandini M; Marconcini L; Ferruzzi R; Oliviero S
Dipartimento di Biologia Molecolare Universita di Siena, Italy. Proceedings of the National Academy of Sciences of the United States of America (UNITED STATES) Oct 15 1996, 93 (21) p11675-80, ISSN 0027-8424 Journal Code: 7505876
Erratum in Proc Natl Acad Sci U S A 1997 Feb 18;94(4) 1603 Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Using a mRNA differential screening of fibroblasts differing for the expression of c-fos we isolated a c-fos-induced growth factor (FIGF). The deduced protein sequence predicts that the cDNA codes for a new member of the platelet-derived growth factor/vascular endothelial growth factor (PDGF/*VEGF*) family. Northern blot analysis shows that FIGF expression is strongly reduced in c-fos-deficient cells. Transfection of exogenous c-fos driven by a constitutive promoter restores the FIGF expression in these cells. In contrast, both PDGF and *VEGF* expression is unaffected by c-fos. FIGF is a secreted *dimeric* protein able to stimulate mitogenic activity in fibroblasts. FIGF overexpression induces morphological alterations in fibroblasts. The cells acquire a spindle-shaped morphology, become more refractive, disorganized, and detach from the plate. These results imply that FIGF is a downstream growth and morphogenic effector of c-fos. These results also suggest that the expression of FIGF in response to c-fos activation induces specific differentiation patterns and its aberrant activation contributes to the malignant phenotype of tumors.

10/3,AB/31 (Item 31 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08903189 96265031 PMID: 8670141
Synthesis and physiological activity of heterodimers comprising different splice forms of vascular endothelial growth factor and placenta growth factor.
Birkenhager R; Schneppe B; Rockl W; Wilting J; Weich H A; McCarthy J E Department of Gene Expression, National Biotechnology Research Centre (GBF), Braunschweig, Federal Republic of Germany.
Biochemical journal (ENGLAND) Jun 15 1996, 316 (Pt 3) p703-7, ISSN 0264-6021 Journal Code: 2984726R
Document type: Journal Article

Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Vascular endothelial growth factor (*VEGF*) and placenta growth factor (PIGF) are members of a *dimeric*-growth-factor family with angiogenic properties. *VEGF* is a highly potent and specific mitogen for endothelial cells, playing a vital role in angiogenesis in vivo. The role of PIGF is less clear. We expressed the monomeric splice forms *VEGF*-165, *VEGF* -121, PIGF-1 and PIGF-2 as unfused genes in Escherichia coli using the pCYTEXP expression system. In vitro dimerization experiments revealed that both homo- and hetero-dimers can be formed from these monomeric proteins. The dimers were tested for their ability to promote capillary growth in vivo and stimulate DNA synthesis in cultured human vascular endothelial cells. Heterodimers comprising different *VEGF* splice forms, or combinations of *VEGF*/PIGF splice forms, showed mitogenic activity. The results demonstrate that four different heterodimeric growth factors are likely to have as yet uncharacterized functions in vivo.

10/3,AB/32 (Item 32 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08847022 96215024 PMID: 8621427
Identification of vascular endothelial growth factor determinants for binding KDR and FLT-1 receptors. Generation of receptor-selective *VEGF* variants by site-directed mutagenesis.
Keyt B A; Nguyen H V; Berleau L T; Duarte C M; Park J; Chen H; Ferrara N Department of Cardiovascular Research, Genentech, Inc., South San Francisco, California 94080, USA.
Journal of biological chemistry (UNITED STATES) Mar 8 1996, 271 (10) p5638-46, ISSN 0021-9258 Journal Code: 2985121R
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Vascular endothelial growth factor (*VEGF*) expression in various cell types is induced by hypoxia and other stimuli. *VEGF* mediates endothelial cell proliferation, angiogenesis, vascular growth, and vascular permeability via the endothelial cell receptors, kinase insert domain-containing receptor (KDR)/fetal liver kinase 1 (Flk-1) and FLT-1. Alanine-scanning mutagenesis was used to identify a positively charged surface in *VEGF* that mediates binding to KDR/Flk-1. Arg82, Lys84 and His86, located in a hairpin loop, were found to be critical for binding KDR/Flk-1, while negatively charged residues, Asp63, Glu64, and Glu67, were associated with FLT-1 binding. A *VEGF* model based on PDGFb indicated these positively and negatively charged regions are distal in the monomer but are spatially close in the *dimer*. Mutations within the KDR site had minimal effect on FLT-1 binding, and mutants deficient in FLT-1 binding did not affect KDR binding. Endothelial cell mitogenesis was abolished in mutants lacking KDR affinity; however, FLT-1 deficient mutants induced normal proliferation. These results suggest dual sets of determinants in the *VEGF* *dimer* that cross-link cell surface receptors, triggering endothelial cell growth and angiogenesis. Furthermore, this mutational analysis implicates KDR, but not FLT-1, in *VEGF* induction of endothelial cell proliferation.

10/3,AB/33 (Item 33 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08779763 96148626 PMID: 8551388
Expression of vascular endothelial growth factor in renal vascular disease and renal allografts.
Grone H J; Simon M; Grone E F
Department of Pathology, University of Marburg, Germany. Journal of pathology (ENGLAND) Nov 1995, 177 (3) p259-67, ISSN 0022-3417 Journal Code: 0204634
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Vascular endothelial growth factor (*VEGF*) is a *dimeric* glycoprotein that exerts a proliferative effect specifically on endothelial cells. *VEGF* can increase vascular permeability and collagenase activity, is chemotactic for monocytes, and may dilate blood vessels. It can be induced by phorbol ester and cAMP in both mesenchymal and epithelial cells. In vitro cell culture experiments suggest that *VEGF* is upregulated by oxygen deprivation. In this study we tested whether in vivo acute and/or chronic reduction of renal blood flow by vascular obstruction would result in increased expression of *VEGF* mRNA and protein. Three normal kidneys, five human kidneys with narrowing of preglomerular vessels by vascular rejection or by vasculitis, and eight kidneys with nephrosclerosis and/or diabetic nephropathy were examined. In situ hybridization with 35S-labelled riboprobes showed a pronounced expression of *VEGF* mRNA in acutely hypoxic proximal and distal tubules of both the cortex and medulla; *VEGF* protein was demonstrated in the epithelia of these tubules by immunohistochemistry. In kidneys with chronically reduced blood flow, the majority of atrophic tubules were negative for *VEGF* mRNA and protein, although interstitial cells expressed *VEGF* mRNA. In arcuate arteries showing intimal and adventitial fibrosis, some medial smooth muscle

cells were positive for *VEGF* mRNA. In glomeruli with segmental sclerosis, viable podocytes showed a prominent signal for *VEGF* mRNA. Mesangial cells did not express *VEGF* in the cases studied. It is possible that hypoxia itself led to the upregulation of *VEGF* in tubular epithelia and vascular smooth muscle cells. The vasodilatory and permeability-promoting effects of the endothelial growth factor produced by damaged tubular epithelia may constitute a mechanism to alleviate a decrease in blood flow and substrate availability and to re-establish vascular integrity.

10/3,AB/34 (Item 34 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08469969 95221439 PMID: 7706320
Purification and characterization of a naturally occurring vascular endothelial growth factor.placenta growth factor heterodimer. DiSalvo J; Bayne M L; Conn G; Kwok P W; Trivedi P G; Soderman D D; Palisi T M; Sullivan K A; Thomas K A
Department of Biochemistry, Merck Research Laboratories, Rahway, New Jersey 07065, USA.
Journal of biological chemistry (UNITED STATES) Mar 31 1995, 270 (13) p7717-23, ISSN 0021-9258 Journal Code: 2985121R
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Vascular endothelial growth factor (*VEGF*) is a potent and selective mitogen for endothelial cells that is angiogenic in vivo and induced by hypoxia. A homologous protein, placenta growth factor (PlGF), is also reported to be mitogenic for endothelial cells in culture. The rat GS-9L glioma cell line produces not only *VEGF* homodimers but also PlGF homodimers and a novel heterodimer composed of *VEGF* and PlGF subunits. All three *dimeric* forms were purified to apparent homogeneity, and their structures and mitogenic activities were compared. *VEGF* .PlGF heterodimers are vascular endothelial cell mitogens nearly as potent as *VEGF* homodimers. Therefore, some of the biological activities attributed to *VEGF* homodimers might be mediated by *VEGF* .PlGF heterodimers. In contrast, pure PlGF homodimers are mitogenic for endothelial cells only at high, possibly non-physiologic concentrations; thus the biological relevance of their mitogenic activity for these cells is not obvious. However, the existence of not only homodimers but also heterodimers clearly extends the similarity between the *VEGF*/PlGF and the homologous platelet-derived growth factor systems.

10/3,AB/35 (Item 35 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08425365 95178508 PMID: 7533000
The shortest isoform of human vascular endothelial growth factor/vascular permeability factor (*VEGF* /VPF121) produced by Saccharomyces cerevisiae promotes both angiogenesis and vascular permeability.
Kondo S; Matsumoto T; Yokoyama Y; Ohmori I; Suzuki H
Bioscience Department, TOAGOSEI Tsukuba Research Lab., Ibaraki, Japan. Biochimica et biophysica acta (NETHERLANDS) Feb 23 1995, 1243 (2) p195-202, ISSN 0006-3002 Journal Code: 0217513
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Vascular endothelial growth factor/vascular permeability factor (*VEGF* /VPF) is a multifunctional cytokine that is expressed as four isoforms having 206, 189, 165, and 121 amino acids in humans. We constructed a system that produces the shortest isoform of human *VEGF* /VPF in Saccharomyces cerevisiae (yVEGF/VPF121). Active yVEGF/VPF121 was secreted from the yeast cells as a glycosylated *dimeric* protein. Various biological activities of the purified yVEGF/VPF121 were examined. It bound to cell surface receptor(s) and stimulated the growth of human umbilical vein endothelial cells in culture at a dose similar to that of native *VEGF* /VPF. Purified yVEGF/VPF121 also induced angiogenesis in mice, and promoted the extravasation of plasma proteins from the blood vessels. These observations demonstrated that the shortest isoform of *VEGF* /VPF with an amino acid sequence of 121 residues contains enough information necessary to trigger both angiogenesis and the induction of vascular permeability upon binding to its cognate receptor(s).

10/3,AB/36 (Item 36 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07814127 93345411 PMID: 8344219
Vascular endothelial growth factor/vascular permeability factor expression in the rat uterus: rapid stimulation by estrogen correlates with estrogen-induced increases in uterine capillary permeability and growth.
Cullinan-Bove K; Koos R D
Department of Physiology, University of Maryland School of Medicine, Baltimore 21201.
Endocrinology (UNITED STATES) Aug 1993, 133 (2) p829-37, ISSN 0013-7227 Journal Code: 0375040
Contract/Grant No.: CA-45055; CA; NCI; HD-07170; HD; NICHD; HD-28055; HD; NICHD
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM

Record type: Completed
In the uterus, estrogen causes a rapid increase in microvascular permeability, followed later by growth of the endometrium, including the richly vascular stroma. Vascular endothelial growth factor/vascular permeability factor (*VEGF* /VPF or VEG/PF) is an angiogenic protein that is not only a specific mitogen for endothelial cells, but also a potent stimulator of microvascular permeability. Because of these properties, it seems likely that VEG/PF might mediate estrogen-induced increases in uterine vascular permeability and blood vessel growth. Therefore, we determined whether the gene for VEG/PF is expressed in the rat uterus and if mRNA abundance is regulated by steroid hormones, using reverse transcription-polymerase chain reaction. The VEG/PF gene is alternatively spliced and gives rise to three transcripts coding for proteins of 188, 164, and 120 amino acids, which, in turn, form the active *dimeric* factors. Transcripts for VEG/PF mRNAs were detected in the uterus of the rat by reverse transcription-polymerase chain reaction. The mRNAs for the VEG/PF164 and VEG/PF120 subunits were the dominant forms expressed. Treatment with both estradiol (E2) and estriol (E3) rapidly induced an increase in the level of the two smaller transcripts. The increase was detectable as early as 0.5-1 h and peaked at 2 h. Levels of the two smaller transcripts then declined, but remained above control levels for 24 h. The degree of stimulation of VEG/PF mRNA levels was 8-fold at 2 h. VEG/PF188 mRNA levels were higher by 6 h compared to control values. The increase in VEG/PF mRNA levels in response to E2 was not contingent upon de novo protein synthesis, as it was not blocked by cycloheximide. The increase occurred as rapidly as that of the mRNA for Zif268, an estrogen-induced transcription factor. Progesterone also stimulated the expression (at 6 h) of VEG/PF164 and VEG/PF120, but not that of VEG/PF188. We conclude that the VEG/PF gene is expressed in the rat uterus, and that mRNA levels are rapidly enhanced by estrogen. This response suggests that VEG/PF may be involved in the estrogen-induced increase in permeability and proliferation of uterine blood vessels. The identification of VEG/PF as a primary response gene also suggests that VEG/PF expression may be a prerequisite for the subsequent expression or action of other growth factors in the uterus.

10/3,AB/37 (Item 37 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07678451 93205407 PMID: 7681160
Two alternative mRNAs coding for the angiogenic factor, placenta growth factor (PlGF), are transcribed from a single gene of chromosome 14. Maglione D; Guerriero V; Viglietto G; Ferraro M G; Aprelikova O; Alitalo K; Del Vecchio S; Lei K J; Chou J Y; Persico M G
International Institute of Genetics and Biophysics, CNR, Naples, Italy. Oncogene (ENGLAND) Apr 1993, 8 (4) p925-31, ISSN 0950-9232 Journal Code: 8711562
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
We have previously reported on the identification of a cDNA (placenta growth factor, PlGF) coding for a novel angiogenic factor expressed in placental tissue that is similar to vascular permeability factor/vascular endothelial growth factor (VPF/*VEGF*). Biochemical and functional characterization of PlGF derived from transfected COS-1 cells revealed that it is a glycosylated *dimeric* secreted protein able to stimulate endothelial cell growth in vitro. Here, we report the isolation and characterization of the PlGF gene located on chromosome 14. At least two different mRNAs are produced from this single-copy gene in different cell lines and tissues. Sequence comparison of the polypeptides encoded by the two different isolated cDNAs indicates that they are identical except for the insertion of a highly basic 21 amino acid stretch at the carboxyl end of the protein. RNA expression analysis of several tissues, tumors and cell lines indicates differential distribution of the two PlGF mRNAs. Finally, preliminary results indicate that the PIGF gene has been conserved in evolution, since the human PlGF cDNA hybridizes to sequences present in the genomic DNA of Drosophila, Xenopus, chicken and mouse.

10/3,AB/38 (Item 38 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07662466 93183008 PMID: 8442657
Inhibition of vascular permeability factor (vascular endothelial growth factor) with antipeptide antibodies.
Sioussat T M; Dvorak H F; Brock T A; Senger D R
Department of Pathology, Beth Israel Hospital, Boston, Massachusetts. Archives of biochemistry and biophysics (UNITED STATES) Feb 15 1993, 301 (1) p15-20, ISSN 0003-9861 Journal Code: 0372430
Contract/Grant No.: CA-28471; CA; NCI; CA-43967; CA; NCI; CA-50453; CA; NCI; +
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Vascular permeability factor (VPF), also known as vascular endothelial cell growth factor (*VEGF*), is a 34- to 43-kDa *dimeric* protein synthesized and secreted by a variety of tumor and normal cells. At

nanomolar concentrations, VPF causes an increase in microvascular permeability and is thought to be responsible for enhanced permeability of tumor blood vessels and for the fluid accumulation associated with solid and ascites tumors. In addition, VPF/*VEGF* is a mitogen for endothelial cells and may play an important role in maintaining vascular endothelium and in promoting tumor angiogenesis. Antibodies were raised against a series of synthetic peptides derived from the predicted human VPF amino acid sequence. The antibodies were assayed for their ability to bind native and denatured/reduced VPF. Antibodies to peptides from the N- and C-termini bound both denatured/reduced and native VPF; antibodies directed to internal segments (e.g., amino acids 27-48 and 85-101) strongly bound denatured/reduced VPF but were substantially less effective at binding native VPF. These results suggest that the N- and C-termini are exposed regions of the protein in solution. Individually, antibodies to the N- and C-termini each partially blocked VPF permeability activity, and, in combination, blocked nearly 100% of this activity. Also, the N- and C-terminal antibodies blocked the VPF-mediated stimulation of both endothelial cell growth and increase in free cytosolic calcium.

10/3,AB/39 (Item 39 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

06501530 90207249 PMID: 2320579
Amino acid and cDNA sequences of a vascular endothelial cell mitogen that is homologous to platelet-derived growth factor.
Conn G; Bayne M L; Soderman D D; Kwok P W; Sullivan K A; Palisi T M; Hope D A; Thomas K A
Department of Biochemistry, Merck Sharp & Dohme Research Laboratories, Rahway, NJ 07065.
Proceedings of the National Academy of Sciences of the United States of America (UNITED STATES) Apr 1990, 87 (7) p2628-32, ISSN 0027-8424 Journal Code: 7505876
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Glioma-derived vascular endothelial cell growth factor (GD-*VEGF*) is a 46-kDa *dimeric* glycoprotein mitogen with apparently greater specificity for vascular endothelial cells than the well-characterized fibroblast growth factors. The GD-*VEGF* cDNA sequence encodes a 190-amino acid residue subunit that is converted, by removal of an amino-terminal hydrophobic secretory leader sequence, to the mature 164-residue subunit characterized by direct amino acid sequencing. The GD- *VEGF* homodimeric subunit is homologous to the platelet-derived growth factor A and B chains and its oncogene homologue v-sis.

10/3,AB/40 (Item 1 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
(c) 2002 Inst for Sci Info. All rts. reserv.

10472213 Genuine Article#: 532AK Number of References: 37 Title: Plasma fibrin D-*dimer* levels correlate with tumour volume, progression rate and survival in patients with metastatic breast cancer (ABSTRACT AVAILABLE)
Author(s): Dirix LY (REPRINT) ; Salgado R; Weytjens R; Colpaert C; Benoy I; Huget P; van Dam P; Prove A; Lemmens J; Vermeulen P
Corporate Source: AZ St Augustinus,Ctr Oncol,Oosterveldlaan 24/B-2610 Wilrijk//Belgium/ (REPRINT); AZ St Augustinus,Ctr Oncol,B-2610 Wilrijk//Belgium/; Univ Antwerp Hosp,Dept Pathol,B-2520 Edegem//Belgium/
Journal: BRITISH JOURNAL OF CANCER, 2002, V86, N3 (FEB 1), P389-395 ISSN: 0007-0920 Publication date: 20020201
Publisher: CHURCHILL LIVINGSTONE, JOURNAL PRODUCTION DEPT, ROBERT STEVENSON HOUSE, 1-3 BAXTERS PLACE, LEITH WALK, EDINBURGH EH1 3AF, MIDLOTHIAN, SCOTLAND
Language: English Document Type: ARTICLE
Abstract: Plasma levels of D-*dimer* are elevated in cancer patients. Activation of the extrinsic coagulation system and the fibrinolytic cascade within a tumour is thought to be related with growth, invasion and metastasis. We have investigated the relationship between these marker's of fibrin metabolism, standard clinicopathological variables and serum levels of angiogenic cytokines in three cohorts: group A (n=30) consisted of 30 healthy female volunteers, group B (n=23) of consecutive patients with operable breast cancer and group C (n=84) of patients with untreated or progressive metastatic breast cancer. Plasma D-dimers, fibrinogen, IL-6, vascular endothelial growth factor and calculated vascular endothelial growth factor load in platelets are clearly increased in patients with breast cancer. D-dimers were increased in nearly 89% of patients with progressive metastatic disease. The level of D-dimers was positively correlated with tumour load ($P < 0.0001$), number of metastatic sites ($P=0.002$), progression kinetics ($P<0.0001$) and the cytokines related to angiogenesis: serum vascular endothelial growth factor ($P=0.0016$, Spearman correlation=0.285), calculated vascular endothelial growth factor load in platelets ($P<0.0001$, Spearman correlation=0.37) and serum interleukin-6 ($P<0.0001$, Spearman correlation=0.59). Similarly increased D-*dimer* levels were positively correlated with increased fibrinogen levels ($P<0.0001$, Spearman correlation=0.38). The

association between markers of fibrin degradation in patients with progressive breast cancer suggests that the D-*dimer* level is a clinically important marker for progression and points towards a relation between haemostasis and tumour progression. A role of interleukin-6, by influencing both angiogenesis and haemostasis, is suggested by these observations.

10/3,AB/41 (Item 2 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
(c) 2002 Inst for Sci Info. All rts. reserv.

09301131 Genuine Article#: 390CM Number of References: 39 Title: Ovarian follicular concentrations of activin, follistatin, inhibin, insulin-like growth factor I (IGF-I), IGF-II, IGF-Binding protein-2 (IGFBP-2), IGFBP-3, and vascular endothelial growth factor in spontaneous menstrual cycles of normal women of advanced reproductive age (ABSTRACT AVAILABLE)
Author(s): Klein NA (REPRINT) ; Battaglia DE; Woodruff TK; Padmanabhan V; Giudice LC; Bremner WJ; Soules MR
Corporate Source: 4225 Roosevelt Way NE,Suite 305/Seattle//WA/98105 (REPRINT); Univ Washington,Seattle//WA/98105; Northwestern Univ,Chicago//IL/60208; Univ Michigan,Ann Arbor//MI/48109; Stanford Univ,Stanford//CA/94305
Journal: JOURNAL OF CLINICAL ENDOCRINOLOGY AND METABOLISM, 2000, V85, N12 (DEC), P4520-4525
ISSN: 0021-972X Publication date: 20001200
Publisher: ENDOCRINE SOC, 4350 EAST WEST HIGHWAY SUITE 500, BETHESDA, MD 20814-4110 USA
Language: English Document Type: ARTICLE
Abstract: Previous studies indicate that the menstrual cycles of older reproductive age women are characterized by a selective elevation of FSH associated with early development and ovulation of a dominant follicle. Several intraovarian hormones and growth factors have been identified that appear to serve important paracrine roles. The purpose of this study was to examine follicular fluid (FF) hormones and growth factors in the dominant follicle of unstimulated cycles of older, ovulatory women. We aspirated FF from the preovulatory dominant follicle in natural menstrual cycles of older subjects (age, 40-45 yr; n = 20) and younger controls (age, 20-25 yr; n = 19). FF was analyzed for estradiol, progesterone, testosterone, androstenedione, inhibin A and B, total activin A, total follistatin, insulin-like growth factor I (IGF-I), IGF-II, IGF-binding protein-2 (IGFBP-2), IGFBP-3, and vascular endothelial growth factor (*VEGF*) concentrations. We found that the dominant follicles from older women contain normal concentrations of steroids, inhibin A and B, IGF-II, IGFBP-2, and IGFBP-3; increased concentrations of follistatin, activin A, and *VEGF*; and decreased concentrations of IGF-I. Therefore, under the influence of elevated FSH, the dominant follicle in older women is highly competent in terms of hormone and growth factor secretion. We postulate that elevated FF activin may be related to the early ovulation observed in older women, whereas elevated *VEGF* may be related to the meiotic spindle abnormalities observed in the oocytes of older reproductive age women.

10/3,AB/42 (Item 3 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
(c) 2002 Inst for Sci Info. All rts. reserv.

09062283 Genuine Article#: 350KR Number of References: 50 Title: Placenta growth factor is induced in human keratinocytes during wound healing (ABSTRACT AVAILABLE)
Author(s): Failla CM; Odorisio T; Cianfarani F; Schietroma C; Puddu P; Zambruno G (REPRINT)
Corporate Source: IRCCS,IDI, INST DERMOPAT IMMACOLATA, MOL CELL BIOL LAB, VIA MONTI DI CRETA 104/I-00167 ROME//ITALY/ (REPRINT); IRCCS,IDI, INST DERMOPAT IMMACOLATA, MOL CELL BIOL LAB/I-00167 ROME//ITALY/; IRCCS,IDI, DEPT IMMUNODERMATOL/ROME//ITALY/
Journal: JOURNAL OF INVESTIGATIVE DERMATOLOGY, 2000, V115, N3 (SEP), P 388-395
ISSN: 0022-202X Publication date: 20000900
Publisher: BLACKWELL SCIENCE INC, 350 MAIN ST, MALDEN, MA 02148 Language: English Document Type: ARTICLE
Abstract: Placenta growth factor (PlGF) is a *dimeric* glycoprotein, structurally and functionally related to the vascular endothelial growth factor, a potent angiogenic/permeability factor known to play a role in the neoangiogenesis during wound repair. In this study we evaluated the expression of PlGF in human keratinocytes and investigated its possible role in wound healing. Northern blot analysis on cultured keratinocytes revealed a 1.7 kb mRNA transcript and reverse transcriptase-polymerase chain reaction allowed the detection of two PlGF isoforms generated by alternative RNA splicing. PlGF and vascular endothelial growth factor homodimers as well as vascular endothelial growth factor/PlGF heterodimers could be detected in keratinocyte conditioned medium. Increased expression of both PlGF mRNA and protein was observed upon treatment of keratinocytes with epidermal growth factor, transforming growth factor-alpha, transforming growth factor-beta, and interleukin-6, all cytokines present at the wound site during the early phase of repair, The analysis of human full-thickness healing wounds revealed appreciable levels of PlGF mRNA and

protein in the migrating keratinocytes starting from day 3 after injury, and increasing at day 5, At day 7 PlGF mRNA was no longer detectable, while the protein was still expressed by migrating suprabasal keratinocytes, At day 13, when the wound had reepithelialized, PlGF immunostaining was completely negative. By in situ hybridization an intense signal for PlGF was also found on endothelial capillaries adjacent to the wound. These data demonstrate that keratinocytes are a source of PlGF during wound healing in vivo and indicate a role for this factor in the neoangiogenesis process associated with cutaneous wound repair.

10/3,AB/43 (Item 4 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
(c) 2002 Inst for Sci Info. All rts. reserv.

08210592 Genuine Article#: 244UC Number of References: 0 Title: Plasma D-*dimer* levels, serum *VEGF*, b-FGF and IL-6 in metastatic breast cancer (MBC): Correlation with tumour load and response to therapy
Author(s): Dirix L; Weytjens R; Salgado R; Benoy I; Vermeulen P; Prove A; VanDam P; Lemmens J
Corporate Source: SINT AUGUSTINUS,/WILRIJK//BELGIUM/
Journal: EUROPEAN JOURNAL OF CANCER, 1999, V35, 4 (SEP), P359-359 ISSN: 0959-8049 Publication date: 19990900
Publisher: PERGAMON-ELSEVIER SCIENCE LTD, THE BOULEVARD, LANGFORD LANE, KIDLINGTON, OXFORD OX5 1GB, ENGLAND
Language: English Document Type: MEETING ABSTRACT

10/3,AB/44 (Item 5 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
(c) 2002 Inst for Sci Info. All rts. reserv.

08033637 Genuine Article#: 239FC Number of References: 42 Title: Vascular endothelial growth factor *VEGF*-like heparin-binding protein from the venom of Vipera aspis aspis (Aspic viper) (ABSTRACT AVAILABLE)
Author(s): Komori Y (REPRINT) ; Nikai T; Taniguchi K; Masuda K; Sugihara H Corporate Source: MEIJO UNIV,FAC PHARM, DEPT MICROBIOL, TENPAKU KU, 150 YAGOTAYAMA/NAGOYA/AICHI 4688503/JAPAN/ (REPRINT); MEIJO UNIV,FAC PHARM, ANALYT CTR, TENPAKU KU/NAGOYA/AICHI 4688503/JAPAN/
Journal: BIOCHEMISTRY, 1999, V38, N36 (SEP 7), P11796-11803 ISSN: 0006-2960 Publication date: 19990907
Publisher: AMER CHEMICAL SOC, 1155 16TH ST, NW, WASHINGTON, DC 20036 Language: English Document Type: ARTICLE
Abstract: The heparin-binding *dimeric* hypotensive factor (HF) was purified from Vipera aspis aspis (Aspic viper) venom [Komori, Y. and Sugihara, H. (1990) Toxicon 28, 359-369]. In this study, the amino acid sequence, and structure and function of HF, were elucidated. By electrospray ionization mass spectrometry (ESI-MS), the molecular weight of HF was determined to be 25 072.1. The complete amino acid sequence of HF was determined by Edman sequencing of the S-pyridylethylated HF and its peptides derived from enzymatic digestion. The theoretical molecular mass calculated from the primary structure agrees well with the molecular weight determined by ESI-MS. HF consists of two homogeneous monomers bound covalently. The monomer with an N-terminal blocked by pyroglutamic acid contains 110 amino acid residues, including eight cysteine residues, two of which are considered to be involved in intermolecular disulfide bonds. Sequential homology search revealed that the primary structure of HF is similar to that of vascular endothelial growth factor (*VEGF*) and platelet-derived growth factor (PDGF) with a sequential homology of 45 and 22%, respectively. When injected intradermally into a rat, an increase in capillary permeability was observed with HF or *VEGF*. On the other hand, only HF exerted a strong hypotensive effect after intravenous injection of samples into a rat. Purified HF has a mitogenic effect on endothelial cells. Through the use of bovine aortic endothelial cells (BAEC), the half-maximal mitogenic concentration of HF was determined to be 5-5.5 nM (125-138 ng/mL). Similarly, *VEGF* had a mitogenic concentration at 0.5-1 nM. When incubated with I-IF and cycloheximide or HF and heparin, the cell growth was inhibited, suggesting that the mechanism of action of HI; is similar to that of *VEGF*.

10/3,AB/45 (Item 6 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
(c) 2002 Inst for Sci Info. All rts. reserv.

07679375 Genuine Article#: 195GY Number of References: 22 Title: Cytokines in older patients undergoing in vitro fertilization: The relationship to the response to controlled ovarian hyperstimulation (ABSTRACT AVAILABLE)
Author(s): Pellicer A (REPRINT) ; Garrido N; Albert C; Navarro J; Remohi J; Simon C
Corporate Source: INST VALENCIANO INFERTIL,GUARDIA CIVIL 23/VALENCIA 46020//SPAIN/ (REPRINT)
Journal: JOURNAL OF ASSISTED REPRODUCTION AND GENETICS, 1999, V16, N5 (MAY) , P247-252

ISSN: 1058-0468 Publication date: 19990500
Publisher: PLENUM PUBL CORP, 233 SPRING ST, NEW YORK, NY 10013 Language: English Document Type: ARTICLE
Abstract: Purpose: Our purpose was to assess the endocrine, autocrine, and paracrine milieu in follicles of older women undergoing stimulated cycles, comparing normal (NR) and low (LR) responses, based on the measurement of interleukin (IL)-1 beta, IL-6, and vascular endothelial growth factor (*VEGF*) in serum and follicular fluid (FF).

Methods: A total of 40 women entered the study, divided into three groups: (1) older patients (>37 years) with NR (age-NR; n = 18); (2) older women with LR (age-LR; n = 11); and (3) normal controls, aged <35 years (control: n = 11). IL-1 beta, IL-6, and *VEGF* measured in serum (day of ovum pickup) and FF, employing ELISAs.

Results: Follicular fluid IL-6 was significantly (P < 0.05) higher in age-LR compared to the other two groups. IL-6 and *VEGF* showed a 4- to 29-fold increase in FF compared to blood, suggesting the ovary as an additional source of both cytokines. IL-1 beta levels remained unchanged in FF compared to blood and, also, among groups.

Conclusions: These data provide further evidence that the endocrine, paracrine, and/or autocrine status in vivo of older patients is different from that of younger women and suggest that cytokines, specifically IL-6, may be involved in the changes observed during senescence within the ovary.

10/3,AB/46 (Item 7 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
(c) 2002 Inst for Sci Info. All rts. reserv.

07409559 Genuine Article#: 162KH Number of References: 65 Title: A novel vascular endothelial growth factor encoded by Orf virus, *VEGF*-E, mediates angiogenesis via signalling through VEGFR-2 (KDR) but not VEGFR-1 (Flt-1) receptor tyrosine kinases (ABSTRACT AVAILABLE)
Author(s): Meyer M; Clauss M; LeppleWienhues A; Waltenberger J; Augustin HG ; Ziche M; Lanz C; Buttner M; Rziha HJ; Dehio C (REPRINT) Corporate Source: MAX PLANCK INST BIOL,DEPT INFECT BIOL, SPEMANNSTR 34/D-72076 TUBINGEN//GERMANY/ (REPRINT); MAX PLANCK INST BIOL,DEPT INFECT BIOL/D-72076 TUBINGEN//GERMANY/; MAX PLANCK INST PHYSIOL & CLIN RES,DEPT MOL CELL BIOL/D-61231 BAD NAUHEIM//GERMANY/; UNIV TUBINGEN,DEPT PHYSIOL/D-72076 TUBINGEN//GERMANY/; UNIV ULM,MED CTR, DEPT INTERNAL MED 2/D-89081 ULM//GERMANY/; UNIV GOTTINGEN,SCH MED, DEPT OBSTET & GYNAECOL/D-37075 GOTTINGEN//GERMANY/; FED RES CTR VIRUS DIS ANIM,INST VACCINES/D-72076 TUBINGEN//GERMANY/; UNIV FLORENCE,DEPT PHARMACOL/I-50134 FLORENCE//ITALY/
Journal: EMBO JOURNAL, 1999, V18, N2 (JAN 15), P363-374
ISSN: 0261-4189 Publication date: 19990115
Publisher: OXFORD UNIV PRESS, GREAT CLARENDON ST, OXFORD OX2 6DP, ENGLAND Language: English Document Type: ARTICLE
Abstract: The different members of the vascular endothelial growth factor (*VEGF*) family act as key regulators of endothelial cell function controlling vasculogenesis, angiogenesis, vascular permeability and endothelial cell survival. In this study, we have functionally characterized a novel member of the *VEGF* family, designated *VEGF*-E. *VEGF*-E sequences are encoded by the para-poxvirus Orf virus (OV). They carry the characteristic cysteine knot motif present in all mammalian VEGFs, while forming a microheterogenic group distinct from previously described members of this family. *VEGF*-E was expressed as the native protein in mammalian cells or as a recombinant protein in Escherichia coil and was shown to act as a heat-stable, secreted *dimer*, *VEGF*-E and *VEGF*-A were found to possess similar bioactivities, i.e. both factors stimulate the release of tissue factor (TF), the proliferation, chemotaxis and sprouting of cultured vascular endothelial cells in vitro and angiogenesis in vivo. Like *VEGF*-A, *VEGF*-E was found to bind with high affinity to *VEGF* receptor-2 (KDR) resulting in receptor autophosphorylation and a biphasic rise in free intracellular Ca2+ concentration, whilst in contrast to *VEGF*-A, *VEGF*-E did not bind to *VEGF* receptor-1 (Flt-1), *VEGF*-E is thus a potent angiogenic factor selectively binding to *VEGF* receptor-2, These data strongly indicate that activation of *VEGF* receptor-2 alone can efficiently stimulate angiogenesis.

10/3,AB/47 (Item 8 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
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07114779 Genuine Article#: 125DM Number of References: 48 Title: Vascular endothelial growth factor B (*VEGF*-B) binds to *VEGF* receptor-1 and regulates plasminogen activator activity in endothelial cells (ABSTRACT AVAILABLE)
Author(s): Olofsson B; Korpelainen E; Pepper MS; Mandriota SJ; Aase K; Kumar V; Gunji Y; Jeltsch MM; Shibuya M; Alitaloi K (REPRINT) ; Eriksson U Corporate Source: HAARTMAN INST,MOL CANC BIOL LAB, POB 21 HAARTMANINKATU 3/HELSINKI

00014//FINLAND/ (REPRINT); HAARTMAN INST,MOL CANC BIOL LAB/HELSINKI 00014//FINLAND/; LUDWIG INST CANC RES,STOCKHOLM BRANCH/S-17177 STOCKHOLM//SWEDEN/; UNIV GENEVA,MED CTR, DEPT MORPHOL/CH-1211 GENEVA 4//SWITZERLAND/; UNIV TOKYO,INST MED SCI, MINATO KU/TOKYO 108//JAPAN/
Journal: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, 1998, V95, N20 (SEP 29), P11709-11714 ISSN: 0027-8424 Publication date: 19980929
Publisher: NATL ACAD SCIENCES, 2101 CONSTITUTION AVE NW, WASHINGTON, DC 20418
Language: English Document Type: ARTICLE
Abstract: The vascular endothelial growth factor (*VEGF*) family has recently expanded by the identification and cloning of three additional members, namely *VEGF*-B, *VEGF*-C, and *VEGF*-D. In this study we demonstrate that *VEGF*-B binds selectively to *VEGF* receptor-1/Flt-1. This binding can be blocked by excess *VEGF*, indicating that the interaction sites on the receptor are at least partially overlapping. Mutating the putative *VEGF* receptor-1/Flt-1 binding determinants Asp(63), Asp(64), and Glu(67) to alanine residues in *VEGF*-B reduced the affinity to *VEGF* receptor-1 but did not abolish binding. Mutational analysis of conserved cysteines contributing to *VEGF*-B *dimer* formation suggest a structural conservation with *VEGF* and platelet-derived growth factor. Proteolytic processing of the 60-kDa *VEGF*-B-186 *dimer* results in a 34-kDa *dimer* containing the receptor-binding epitopes. The binding of *VEGF*-B to its receptor on endothelial cells leads to increased expression and activity of urokinase type plasminogen activator and plasminogen activator inhibitor 1, suggesting a role for *VEGF*-B in the regulation of extracellular matrix degradation, cell adhesion, and migration.

10/3,AB/48 (Item 9 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
(c) 2002 Inst for Sci Info. All rts. reserv.

07078865 Genuine Article#: 121RR Number of References: 56 Title: *VEGF* and the Fab fragment of a humanized neutralizing antibody: crystal structure of the complex at 2.4 angstrom resolution and mutational analysis of the interface (ABSTRACT AVAILABLE)
Author(s): Muller YA; Chen Y; Christinger HW; Li B; Cunningham BC; Lowman HB; deVos AM (REPRINT)
Corporate Source: GENENTECH INC,DEPT PROT ENGN, 1 DNA WAY/S SAN FRANCISCO//CA/94080 (REPRINT); GENENTECH INC,DEPT PROT ENGN/S SAN FRANCISCO//CA/94080; MAX DELBRUCK CTR MOL MED,FORSCH GRP KRISTALLOG/D-13122 BERLIN//GERMANY/
Journal: STRUCTURE, 1998, V6, N9 (SEP 15), P1153-1167 ISSN: 0969-2126 Publication date: 19980915
Publisher: CURRENT BIOLOGY LTD, 34-42 CLEVELAND STREET, LONDON W1P 6LB, ENGLAND
Language: English Document Type: ARTICLE
Abstract: Background: Vascular endothelial growth factor (*VEGF*) is a highly specific angiogenic growth factor; anti-angiogenic treatment through inhibition of receptor activation by *VEGF* might have important therapeutic applications in diseases such as diabetic retinopathy and cancer. A neutralizing anti-*VEGF* antibody shown to suppress tumor growth in an in vivo murine model has been used as the basis for production of a humanized version.

Results: We present the crystal structure of the complex between *VEGF* and the Fab fragment of this humanized antibody, as well as a comprehensive alanine-scanning analysis of the contact residues on both sides of the interface. Although the *VEGF* residues critical for antibody binding are distinct from those important for high-affinity receptor binding, they occupy a common region an *VEGF*, demonstrating that the neutralizing effect of antibody binding results from steric blocking of *VEGF*-receptor interactions. Of the residues buried in the *VEGF*-Fab interface, only a small number are critical for high-affinity binding; the essential *VEGF* residues interact with those of the Fab fragment, generating a remarkable functional complementarity at the interface.

Conclusions: Our findings suggest that the character of antigen-antibody interfaces is similar to that of other protein-protein interfaces, such as ligand-receptor interactions; in the case of *VEGF*, the principal difference is that the residues essential for binding to the Fab fragment are concentrated in one continuous segment of polypeptide chain, whereas those essential for binding to the receptor are distributed over four different segments and span across the *dimer* interface.

10/3,AB/49 (Item 10 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
(c) 2002 Inst for Sci Info. All rts. reserv.

06314239 Genuine Article#: YH960 Number of References: 41 Title: Crystal structure at 1.7 angstrom resolution of *VEGF* in complex with domain 2 of the Flt-1 receptor (ABSTRACT AVAILABLE)
Author(s): Wiesmann C; Fuh G; Christinger HW; Eigenbrot C; Wells JA; deVos AM (REPRINT)
Corporate Source: GENENTECH INC,DEPT PROT ENGN/S SAN FRANCISCO//CA/94080 (REPRINT); GENENTECH INC,DEPT PROT ENGN/S SAN

FRANCISCO//CA/94080 Journal: CELL, 1997, V91, N5 (NOV 28), P695-704
ISSN: 0092-8674 Publication date: 19971128
Publisher: CELL PRESS, 1050 MASSACHUSETTES AVE, CIRCULATION DEPT, CAMBRIDGE, MA 02138
Language: English Document Type: ARTICLE
Abstract: Vascular endothelial growth factor (*VEGF*) is a homodimeric hormone that induces proliferation of endothelial cells through binding to the kinase domain receptor and the Fms-like tyrosine kinase receptor (Flt-1), the extracellular portions of which consist of seven immunoglobulin domains. We show that the second and third domains of Flt-1 are necessary and sufficient for binding *VEGF* with near-native affinity, and that domain 2 alone binds only 60-fold less tightly than wild-type. The crystal structure of the complex between *VEGF* and the second domain of Flt-1 shows domain 2 in a predominantly hydrophobic interaction with the ''poles'' of the *VEGF* *dimer*. Based on this structure and on mutational data, we present a model of *VEGF* bound to the first four domains of Flt-1.

10/3,AB/50 (Item 11 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
(c) 2002 Inst for Sci Info. All rts. reserv.

05901375 Genuine Article#: XF220 Number of References: 69 Title: Involvement of interleukin-8, vascular endothelial growth factor, and basic fibroblast growth factor in tumor necrosis factor alpha-dependent angiogenesis (ABSTRACT AVAILABLE)
Author(s): Yoshida S; Ono M; Shono T; Izumi H; Ishibashi T; Suzuki H; Kuwano M (REPRINT)
Corporate Source: KYUSHU UNIV,SCH MED, DEPT BIOCHEM/FUKUOKA 81282//JAPAN/ (REPRINT); KYUSHU UNIV,SCH MED, DEPT BIOCHEM/FUKUOKA 81282//JAPAN/; KYUSHU UNIV,SCH MED, DEPT OPHTHALMOL/FUKUOKA 81282//JAPAN/; TOAGOSEI CO LTD,TSUKUBA RES LAB/IBARAKI/OSAKA 30033/JAPAN/
Journal: MOLECULAR AND CELLULAR BIOLOGY, 1997, V17, N7 (JUL), P4015-4023 ISSN: 0270-7306
Publication date: 19970700
Publisher: AMER SOC MICROBIOLOGY, 1325 MASSACHUSETTS AVENUE, NW, WASHINGTON, DC 20005-4171
Language: English Document Type: ARTICLE
Abstract: Tumor necrosis factor alpha (TNF-alpha) is a macrophage/monocyte-derived polypeptide which modulates the expression of various genes in vascular endothelial cells and induces angiogenesis. However, the underlying mechanism by which TNF-alpha mediates angiogenesis is not completely understood, In this study, we assessed whether TNF-alpha-induced angiogenesis is mediated through TNF-alpha itself or indirectly through other TNF-alpha-induced angiogenesis-promoting factors. Cellular mRNA levels of interleukin-8 (IL-8), vascular endothelial growth factor (*VEGF*), basic fibroblast growth factor (bFGF), and their receptors were increased after the treatment of human microvascular endothelial cells with TNF-alpha (100 U/ml). TNF-alpha-dependent tubular morphogenesis in vascular endothelial cells was inhibited by the administration of anti-IL-8, anti-*VEGF*, and anti-bFGF antibodies, and coadministration of all three antibodies almost completely abrogated tubular formation. Moreover, treatment with Spl, NF-kappa B, and c-Jun antisense oligonucleotides inhibited TNF-alpha-dependent tubular morphogenesis by microvascular endothelial cells, Administration of a NF-kappa B antisense oligonucleotide almost completely inhibited TNF-alpha-dependent IL-8 production and partially abrogated TNF-alpha-dependent *VEGF* production, and an Spl antisense sequence partially inhibited TNF-alpha-dependent production of *VEGF*. A c-Jun antisense oligonucleotide significantly inhibited TNF-alpha-dependent bFGF production but did not affect the production of IL-8 and *VEGF*. Administration of an anti-IL-8 or anti-*VEGF* antibody also blocked TNF-alpha-induced neovascularization in the rabbit cornea in vivo. Thus, angiogenesis by TNF-alpha appears to be modulated through various angiogenic factors, both in vitro and in vivo, and this pathway is controlled through paracrine and/or autocrine mechanisms.

10/3,AB/51 (Item 12 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
(c) 2002 Inst for Sci Info. All rts. reserv.

05815450 Genuine Article#: WZ216 Number of References: 37 Title: Inhibition of vascular endothelial growth factor-induced endothelial cell migration by ETS1 antisense oligonucleotides (ABSTRACT AVAILABLE)
Author(s): Chen ZQ; Fisher RJ; Riggs CW; Rhim JS; Lautenberger JA (REPRINT)
Corporate Source: NCI,FREDERICK CANC RES & DEV CTR, SCI APPLICAT INT CORP, POB B, BLDG 560, ROOM /FREDERICK//MD/21702 (REPRINT); NCI,FREDERICK CANC RES & DEV CTR, SCI APPLICAT INT CORP/FREDERICK//MD/21702; NCI,FREDERICK CANC RES & DEV CTR, DATA MANAGEMENT SERV/FREDERICK//MD/21702; NCI,FREDERICK CANC RES & DEV CTR, LAB BIOCHEM PHYSIOL/FREDERICK//MD/21702; NCI,FREDERICK CANC RES & DEV CTR, LAB GENOM DIVERS/FREDERICK//MD/21702
Journal: CANCER RESEARCH, 1997, V57, N10 (MAY 15), P2013-2019 ISSN: 0008-5472 Publication date:

19970515
Publisher: AMER ASSOC CANCER RESEARCH, PUBLIC LEDGER BLDG, SUITE 816, 150 S. INDEPENDENCE MALL W., PHILADELPHIA, PA 19106
Language: English Document Type: ARTICLE
Abstract: Vascular endothelial growth factor (*VEGF*) increased the level of ETS1 mRNA in human umbilical vein endothelial cells (HUVEC) and human lung microvascular endothelial cells (HMVEC-L) over 5-fold. Protein levels were shown to increase concordantly. *VEGF* was also found to stimulate the invasiveness of endothelial cells as measured by migration through Matrigel- or gelatin-coated membranes. The *VEGF* -induced invasiveness was inhibited by ETS1 antisense oligonucleotides but not by a sense control. In addition, the ETS1 antisense oligonucleotides reduced the levels of ETS1 and urokinase-type plasminogen activator mRNAs. The antisense oligonucleotides directed against the ETS1 gene thus altered a cellular property of endothelial cells that is correlated with the ability of the cells to migrate through basement membranes. Together, these observations demonstrate a direct role for the ETS1 gene in angiogenesis.

10/3,AB/52 (Item 13 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
(c) 2002 Inst for Sci Info. All rts. reserv.

05498716 Genuine Article#: WC554 Number of References: 30 Title: *VEGF*-C RECEPTOR-BINDING AND PATTERN OF EXPRESSION WITH VEGFR-3 SUGGESTS A ROLE IN LYMPHATIC VASCULAR DEVELOPMENT (Abstract Available) Author(s): KUKK E; LYMBOUSSAKI A; TAIRA S; KAIPAINEN A; JELTSCH M; JOUKOV V ; ALITALO K
Corporate Source: UNIV HELSINKI,MOL CANC BIOL LAB,HAARTMAN INST,PL21 HAARMANINKATU 3/FIN-00014 HELSINKI//FINLAND/; UNIV HELSINKI,MOL CANC BIOL LAB,HAARTMAN INST/FIN-00014 HELSINKI//FINLAND/
Journal: DEVELOPMENT, 1996, V122, N12 (DEC), P3829-3837
ISSN: 0950-1991
Language: ENGLISH Document Type: ARTICLE
Abstract: The vascular endothelial growth factor family has recently been expanded by the isolation of two new *VEGF*-related factors, *VEGF*-B and *VEGF*-C. The physiological functions of these factors are largely unknown. Here we report the cloning and characterization of mouse *VEGF*-C, which is produced as a disulfide-linked *dimer* of 415 amino acid residue polypeptides, sharing an 85% identity with the human *VEGF*-C amino acid sequence. The recombinant mouse *VEGF*-C protein was secreted from transfected cells as VEGFR-3 (Flt4) binding polypeptides of 30-32x10(3) M(r) and 22-23x10(3) M(r) which preferentially stimulated the autophosphorylation of VEGFR-3 in comparison with VEGFR-2 (KDR), In in situ hybridization, mouse *VEGF*-C mRNA expression was detected in mesenchymal cells of postimplantation mouse embryos, particularly in the regions where the lymphatic vessels undergo sprouting from embryonic veins, such as the perimetanephric, axillary and jugular regions, In addition, the developing mesenterium, which is rich in lymphatic vessels, showed strong *VEGF*-C expression, *VEGF*-C was also highly expressed in adult mouse lung, heart and kidney, where VEGFR-3 was also prominent, The pattern of expression of *VEGF*-C in relation to its major receptor VEGFR-3 during the sprouting of the lymphatic endothelium in embryos suggests a paracrine mode of action and that one of the functions of *VEGF*-C may be in the regulation of angiogenesis of the lymphatic vasculature.

10/3,AB/53 (Item 14 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
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05221035 Genuine Article#: VJ286 Number of References: 34 Title: ACTIVATION-INDUCED EXPRESSION OF VASCULAR-PERMEABILITY FACTOR BY HUMAN PERIPHERAL T-CELLS - A NONRADIOISOTOPIC SEMIQUANTITATIVE REVERSE TRANSCRIPTION-POLYMERASE CHAIN-REACTION ASSAY (Abstract Available) Author(s): IIJIMA K; YOSHIKAWA N; NAKAMURA H
Corporate Source: KOBE UNIV,SCH MED,DEPT PEDIAT,CHUO KU,5-2 KUSUNOKI CHO 7 CHOME/KOBE/HYOGO 650/JAPAN/
Journal: JOURNAL OF IMMUNOLOGICAL METHODS, 1996, V196, N2 (SEP 27), P 199-209
ISSN: 0022-1759
Language: ENGLISH Document Type: ARTICLE
Abstract: Vascular permeability factor, also known as vascular endothelial growth factor (VPF/*VEGF*), is a disulfide-linked *dimeric* glycoprotein of about 40 kDa that enhances vascular permeability, induces chemotaxis and activation of monocytes/macrophages, and promotes growth of vascular endothelial cells. It has been reported that several tumor cell lines and normal cells produce VPF/*VEGF*. To examine the possibility of VPF/*VEGF* mRNA expression in human peripheral T cells and its mechanism(s) of regulation, we developed a non-radioisotopic semiquantitative reverse transcription-polymerase chain reaction (RT-PCR; VPF/*VEGF*, GAPDH co-amplification) assay which detects all species of VPF/*VEGF* mRNA alternative splicing products. T cells expressed negligible VPF/*VEGF* mRNA in the resting state. However, TPA markedly enhanced the expression of 121-, 165- and

189-amino-acid-containing isoforms of VPF/*VEGF* mRNA in T cells. Both CD4(+) and CD8(+) T cells expressed VPF/*VEGF* mRNA in response to TPA treatment. Moreover, T cell receptor stimulation with monoclonal anti-CD5 antibody with or without IL-1 beta enhanced VPF/ *VEGF* mRNA expression in T cells. These findings suggest that T cell activation induces VPF/*VEGF* expression in the cells, resulting in induction of its biological activities.

10/3,AB/54 (Item 15 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
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04852868 Genuine Article#: UM277 Number of References: 16 Title: EXPRESSION OF BIOLOGICALLY-ACTIVE ISOFORMS OF THE TUMOR ANGIOGENESIS FACTOR *VEGF* IN ESCHERICHIA-COLI (Abstract Available) Author(s): SIEMEISTER G; SCHNURR B; MOHRS K; SCHACHTELE C; MARME D; MARTINYBARON G
Corporate Source: INST MOLEC MED,TUMOR BIOL CTR,BREISACHER STR 117/D-79106 FREIBURG//GERMANY/
Journal: BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, 1996, V222, N2 (MAY 15), P249-255
ISSN: 0006-291X
Language: ENGLISH Document Type: ARTICLE
Abstract: Vascular endothelial growth factor (*VEGF*) was identified as an endothelial cell specific mitogen that induces angiogenesis and vascular permeability in vivo. *VEGF* is a homodimeric protein which contains three intramolecular and two intermolecular disulfide bridges. Here, we report on an efficient procedure for recombinant production of *VEGF* isoforms *VEGF*(121) and *VEGF*(165) in Escherichia coli. The proteins were solubilized from inclusion bodies, refolded, and purified by chromatographic methods. The final protein products were almost completely in the *dimeric* conformation, bound to *VEGF* receptor FLT1 with a K-d of 30 pM, stimulated proliferation of human umbilical vein endothelial cells half-maximally at a concentration of 30 pM, and induced in vivo neovascularization and vascular permeability on the chicken chorioallantoic membrane. (C) 1996 Academic Press, Inc.

10/3,AB/55 (Item 16 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
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04206930 Genuine Article#: RN617 Number of References: 51 Title: EXPRESSION OF BIOLOGICALLY-ACTIVE HUMAN VASCULAR ENDOTHELIAL GROWTH-FACTOR IN YEAST (Abstract Available)
Author(s): MOHANRAJ D; OLSON T; RAMAKRISHNAN S
Corporate Source: UNIV MINNESOTA,DEPT PHARMACOL,3-249 MILLARD HALL,435 DELAWARE ST SE/MINNEAPOLIS//MN/55455; UNIV MINNESOTA,DEPT PHARMACOL/MINNEAPOLIS//MN/55455
Journal: GROWTH FACTORS, 1995, V12, N1, P17-27
ISSN: 0897-7194
Language: ENGLISH Document Type: ARTICLE
Abstract: Vascular endothelial growth factor (*VEGF*) is a glycoprotein consisting of two identical polypeptide chains linked by a disulfide bond. The unique biological activities of *VEGF* include its potent mitogenic and permeability inducing properties specific for the vascular endothelium. *VEGF* is implicated in tumor angiogenesis, wound healing, and the stimulation of collateral vessel formation at the site of arterial occlusion. Therefore, in order to produce large quantities of biologically active *VEGF*, a splice variant (*VEGF*(165)) was cloned and expressed in a yeast expression system. The coding region of *VEGF*(165) was isolated from U937 cells by RT-PCR, sequenced and then cloned into the yeast expression vector pHILS1. *VEGF*(165) was secreted into the medium as a *dimer*. Recombinant *VEGF* reacted to antibodies raised against the N-terminal and C-terminal synthetic polypeptides of human *VEGF*. As much as 35-40 mg/L of purified *VEGF* could be obtained from the yeast expression system. The recombinant protein was biologically active in inducing vascular endothelial cell proliferation in vitro and permeability changes in vivo.

10/3,AB/56 (Item 17 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
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03728012 Genuine Article#: QA913 Number of References: 43 Title: STRUCTURAL REQUIREMENTS FOR DIMERIZATION, GLYCOSYLATION, SECRETION, AND BIOLOGICAL FUNCTION OF VPF/*VEGF* (Abstract Available) Author(s): CLAFFEY KP; SENGER DR; SPIEGELMAN BM
Corporate Source: BETH ISRAEL HOSP,DEPT PATHOL,330 BROOKLINE AVE/BOSTON//MA/02215; HARVARD UNIV,SCH MED/BOSTON//MA/02215; HARVARD UNIV,DANA FARBER CANC INST/BOSTON//MA/02115; HARVARD UNIV,DEPT BIOL CHEM & MOLEC PHARMACOL/BOSTON//MA/02115
Journal: BIOCHIMICA ET BIOPHYSICA ACTA-PROTEIN STRUCTURE AND MOLECULAR ENZYMOLOGY, 1995, V1246, N1 (JAN 5), P1-9

ISSN: 0167-4838
Language: ENGLISH Document Type: ARTICLE
Abstract: Vascular permeability factor (VPF) also known as vascular endothelial growth factor (*VEGF*), is a *dimeric* protein that affects endothelial cell (EC) and vascular functions including enhancement of microvascular permeability and stimulation of EC growth. To investigate the structural features of VPF/*VEGF* necessary for efficient dimerization, secretion, and biological activities, we employed site-directed mutagenesis with a Cos-1 cell expression system. Several cysteine residues essential for VPF dimerization were identified by mutation analysis of the Cys-25, Cys-56, and Cys-67 residues. Mutant VPF isoforms lacking either of these cysteines were secreted as monomers and were completely inactive in both vascular permeability and endothelial cell mitotic assays. VPF Cys-145 mutant protein was efficiently secreted as a glycosylated, *dimeric* polypeptide, but had a reduction in biological activities. The site of N-linked glycosylation was directly identified as Asn-74, which, when mutated produced an inefficiently secreted *dimeric* protein without post-translational glycosylation, yet maintained full vascular permeability activity. Finally, we found that one VPF mutant isoform Cys-101 was not secreted and this mutant functioned as a dominant-negative suppressor of wild-type VPF secretion as demonstrated by co-expression assays in Cos-1 cells.

10/3,AB/57 (Item 18 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
(c) 2002 Inst for Sci Info. All rts. reserv.

03724293 Genuine Article#: QA638 Number of References: 37 Title: COVALENT DIMERIZATION OF VASCULAR-PERMEABILITY FACTOR VASCULAR ENDOTHELIAL GROWTH-FACTOR IS ESSENTIAL FOR ITS BIOLOGICAL-ACTIVITY - EVIDENCE FROM CYS TO SER MUTATIONS (Abstract Available) Author(s): POTGENS AJG; LUBSEN NH; VANALTENA MC; VERMEULEN R; BAKKER A; SCHOENMAKERS JGG; RUITER DJ; DEWAAL RMW
Corporate Source: UNIV NIJMEGEN HOSP,INST PATHOL,POB 9101/6500 HBNIJMEGEN//NETHERLANDS/; UNIV NIJMEGEN HOSP,INST PATHOL/6500 HB NIJMEGEN//NETHERLANDS/; UNIV NIJMEGEN,DEPT BIOL MOLEC/NIJMEGEN//NETHERLANDS/
Journal: JOURNAL OF BIOLOGICAL CHEMISTRY, 1994, V269, N52 (DEC 30), P 32879-32885
ISSN: 0021-9258
Language: ENGLISH Document Type: ARTICLE
Abstract: Vascular permeability factor, or vascular endothelial growth factor (VPF/*VEGF*) is an important factor in the regulation of vascular growth and vascular permeability. VPF is a secreted, *dimeric* protein and has 8 cysteine residues conserved with platelet-derived growth factor (PDGF). To study the role of some of these cysteine residues in maintaining the structure and function of VPF, we replaced the codons for the second, third, fourth, and fifth cysteine by serine codons, and expressed the mutant proteins in a mammalian expression system. Cysteine residues 2 and 4 in VPF were found to be directly involved in anti parallel interchain disulfide bonds, as in PDGF. VPF mutants lacking one of these cysteines were severely impaired in their S-linked dimerization, while upon coexpression of both mutants the ability to form dimers was restored. The VPF mutants lacking cysteine residue 2 or 4 also competed poorly for receptor binding of labeled VPF and had low biological activity, but these defects were also complemented by coexpressing the two mutants, indicating that for efficient receptor binding and activation VPF needs to be a covalent *dimer*, unlike PDGP-BB. Furthermore, cysteine residue 5 was found to be essential for VPF dimerization and activity, while the mutant lacking cysteine residue 3 was only mildly affected in its ability to dimerize and had partial biological activity.

10/3,AB/58 (Item 19 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
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02777055 Genuine Article#: MB448 Number of References: 29 Title: HUMAN MESANGIAL CELLS AND PERIPHERAL-BLOOD MONONUCLEAR-CELLS PRODUCE VASCULAR-PERMEABILITY FACTOR (Abstract Available)
Author(s): IIJIMA K; YOSHIKAWA N; CONNOLLY DT; NAKAMURA H
Corporate Source: KOBE UNIV,SCH MED,DEPT PEDIAT,5-2 KUSUNOKI CHO 7 CHOME,CHUO KU/KOBE 650//JAPAN/; MONSANTO RES CORP,DEPT HLTH SCI/ST LOUIS//MO/63166
Journal: KIDNEY INTERNATIONAL, 1993, V44, N5 (NOV), P959-966 ISSN: 0085-2538
Language: ENGLISH Document Type: ARTICLE
Abstract: Vascular permeability factor, or vascular endothelial growth factor (VPF/*VEGF*) is a disulfide-linked *dimeric* glycoprotein of about 40 kD that promotes fluid and protein leakage from blood vessels. Various human tumor cell lines and cells including fetal vascular smooth muscle cells produce VPF/*VEGF*. Since glomerular mesangial cells (MC) are closely related to vascular smooth muscle cells, we examined whether cultured human MC produce VPF/ *VEGF*. Northern blotting analysis revealed that cultured human MC expressed a 3.7 kilobases (kb) VPF/*VEGF* mRNA.

Human peripheral blood mononuclear cells (PBMC) also expressed VPF/*VEGF* transcripts of 8.6 and 3.8 kb. Although the sizes of the transcripts suggested the existence of unique molecular species of VPF/*VEGF* mRNA in PBMC, RT-PCR analysis revealed that PBMC as well as human MC expressed 121, 165, and 189 amino acid-containing isoforms of VPF/ *VEGF*, implying that there are no unique alternative splicing products of VPF/*VEGF* mRNA in PBMC. Fetal calf serum 12-o-tetradecanoyl-phorbol-13-acetate (TPA) transiently enhanced VPF/ *VEGF* mRNA expression in cultured human MC. Transforming growth factor-beta1 enhanced VPF/*VEGF* mRNA expression in cultured human MC at least within 24 hours. Dexamethasone (DEX) inhibited the TPA-induced increase in VPF/*VEGF* mRNA expression, whereas DEX did not change the basal level. That DEX depressed the TPA-induced increase in VPF/*VEGF* mRNA expression is therefore probably a result of transcriptional control. VPF/*VEGF* protein was detected in cultured human MC with immunoperoxidase staining using anti-VPF/ *VEGF* antibody. TPA increased VPF/*VEGF* protein levels as well as those of VPF/*VEGF* mRNA in cultured human MC. These findings indicate that cultured human MC and PBMC produce VPF/ *VEGF* and that it is modulated by various agents. Since VPF/ *VEGF* promotes growth in vascular endothelial cells and enhances vascular permeability, VPF/*VEGF* produced by MC and PBMC may induce the proliferation of glomerular endothelial cells or enhance the permeability of glomerular capillaries.

10/3,AB/59 (Item 20 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
(c) 2002 Inst for Sci Info. All rts. reserv.

02716150 Genuine Article#: LZ105 Number of References: 89 Title: VASCULAR-PERMEABILITY FACTOR (VPF, *VEGF*) IN TUMOR BIOLOGY (Abstract Available)
Author(s): SENGER DR; VANDEWATER L; BROWN LF; NAGY JA; YEO KT; YEO TK; BERSE B; JACKMAN RW; DVORAK AM; DVORAK HF
Corporate Source: BETH ISRAEL HOSP,DEPT PATHOL,330 BROOKLINE AVE/BOSTON//MA/02215; HARVARD UNIV,SCH MED,DEPT PATHOL/BOSTON//MA/02115 Journal: CANCER AND METASTASIS REVIEWS, 1993, V12, N3-4 (SEP), P303-324 ISSN: 0167-7659
Language: ENGLISH Document Type: REVIEW
Abstract: Vascular permeability factor (VPF), also known as vascular endothelial growth factor (*VEGF*), is a multifunctional cytokine expressed and secreted at high levels by many tumor cells of animal and human origin. As secreted by tumor cells, VPF/*VEGF* is a 34-42 kDa heparin-binding, *dimeric*, disulfide-bonded glycoprotein that acts directly on endothelial cells (EC) by way of specific receptors to activate phospholipase C and induce [Ca2+]i transients. Two high affinity VPF/*VEGF* receptors, both tyrosine kinases, have thus far been described. VPF/*VEGF* is likely to have a number of important roles in tumor biology related, but not limited to, the process of tumor angiogenesis. As a potent permeability factor, VPF/*VEGF* promotes extravasation of plasma fibrinogen, leading to fibrin deposition which alters the tumor extracellular matrix. This matrix promotes the ingrowth of macrophages, fibroblasts, and endothelial cells. Moreover, VPF/*VEGF* is a selective endothelial cell (EC) growth factor in vitro, and it presumably stimulates EC proliferation in vivo. Furthermore, VPF/*VEGF* has been found in animal and human tumor effusions by immunoassay and by functional assays and very likely accounts for the induction of malignant ascites. In addition to its role in tumors, VPF/*VEGF* has recently been found to have a role in wound healing and its expression by activated macrophages suggests that it probably also participates in certain types of chronic inflammation. VPF/*VEGF* is expressed in normal development and in certain normal adult organs, notably kidney, heart, adrenal gland and lung. Its functions in normal adult tissues are under investigation.

10/3,AB/60 (Item 21 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
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02312478 Genuine Article#: KT220 Number of References: 35 Title: 2 ALTERNATIVE MESSENGER-RNAS CODING FOR THE ANGIOGENIC FACTOR, PLACENTA GROWTH-FACTOR (PIGF), ARE TRANSCRIBED FROM A SINGLE GENE OF CHROMOSOME-14 (Abstract Available)
Author(s): MAGLIONE D; GUERRIERO V; VIGLIETTO G; FERRARO MG; APRELIKOVA O; ALITALO K; DELVECCHIO S; LEI KJ; CHOU JY; PERSICO MG
Corporate Source: CNR,INT INST GENET & BIOPHYS,VIA G MARCONI 12/I-80125 NAPLES//ITALY/; CNR,INT INST GENET & BIOPHYS,VIA G MARCONI 12/I-80125 NAPLES//ITALY/; SIFI SPA/I-95020 LAVINAIO//ITALY/; UNIV HELSINKI,DEPT PATHOL/SF-00290 HELSINKI 29//FINLAND/; FDN G PASCALE,IST NAZL STUDIO CURA TUMORI/I-80131 NAPLES//ITALY/; NICHHD,HUMAN GENET LAB/BETHESDA//MD/20892
Journal: ONCOGENE, 1993, V8, N4 (APR), P925-931 ISSN: 0950-9232
Language: ENGLISH Document Type: ARTICLE
Abstract: We have previously reported on the identification of a cDNA (placenta growth factor,

PIGF) coding for a novel angiogenic factor expressed in placental tissue that is similar to vascular permeability factor/vascular endothelial growth factor (VPF/*VEGF*). Biochemical and functional characterization of PIGF derived from transfected COS-1 celts revealed that it is a glycosylated *dimeric* secreted protein able to stimulate endothelial cell growth in vitro. Here, we report the isolation and characterization of the PIGF gene located on chromosome 14. At least two different mRNAs are produced from this single-copy gene in different cell lines and tissues. Sequence comparison of the polypeptides encoded by the two different isolated cDNAs indicates that they are identical except for the insertion of a highly basic 21 amino acid stretch at the carboxyl end of the protein. RNA expression analysis of several tissues, tumors and cell tines indicates differential distribution of the two PlGF mRNAs. Finally, preliminary results indicate that the PlGF gene has been conserved in evolution, since the human PlGF cDNA hybridizes to sequences present in the genomic DNA of Drosophila, Xenopus, chicken and mouse.

10/3,AB/61 (Item 22 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
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02193817 Genuine Article#: KH627 Number of References: 41 Title: SYNTHESIS AND ASSEMBLY OF FUNCTIONALLY ACTIVE HUMAN VASCULAR ENDOTHELIAL GROWTH-FACTOR HOMODIMERS IN INSECT CELLS (Abstract Available)
Author(s): FIEBICH BL; JAGER B; SCHOLLMANN C; WEINDEL K; WILTING J; KOCHS G ; MARME D; HUG H; WEICH HA
Corporate Source: UNIV FREIBURG,INST MOLEC CELL BIOL,MOOSWALDALLEE 1-9/W-7800 FREIBURG//GERMANY/; UNIV FREIBURG,INST MOLEC CELL BIOL,MOOSWALDALLEE 1-9/W-7800 FREIBURG//GERMANY/; UNIV FREIBURG,GODECKE AG,INST MOLEC CELL BIOL/W-7800 FREIBURG//GERMANY/; UNIV FREIBURG,INST ANAT/W-7800 FREIBURG//GERMANY/
Journal: EUROPEAN JOURNAL OF BIOCHEMISTRY, 1993, V211, N1-2 (JAN 15), P 19-26
ISSN: 0014-2956
Language: ENGLISH Document Type: ARTICLE
Abstract: Vascular endothelial growth factor (*VEGF*) is an angiogenic growth factor with a target-cell specificity highly restricted to vascular endothelial cells. Recombinant baculovirus were constructed for the production of two different forms of the human *VEGF* protein in insect cells. VEGF165 and VEGF121 proteins produced by Sf158 cells under went a similar processing compared with mammalian cells, including efficient glycosylation, formation of a disulfide-linked *dimer* and secretion into the media. Only one of these forms, VEGF165 had a high affinity for heparin and this characteristic was used to purify this form to homogenicity by a two-step heparin-affinity chromatography. The biological activity of the purified 43-kDa homodimer was demonstrated by high-affinity binding to *VEGF* receptors, and by the induction of DNA synthesis in vascular endothelial cells. A positive angiogenic activity in vivo was demonstrated by the day-13 chorioallantoic-membrane assay. The mitogenic potency of VEGF121 for human umbilical vein endothelial cells was very similar compared to VEGF165. These results demonstrate that an angiogenic growth factor whose normal processing requires glycosylation and disulfide-bridge formation can be efficiently expressed in high concentration (up to 5 mug/ml) in insects cells.

10/3,AB/62 (Item 23 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
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02127368 Genuine Article#: KD073 Number of References: 55 Title: DUAL REGULATION OF VASCULAR ENDOTHELIAL GROWTH-FACTOR BIOAVAILABILITY BY GENETIC AND PROTEOLYTIC MECHANISMS (Abstract Available)
Author(s): HOUCK KA; LEUNG DW; ROWLAND AM; WINER J; FERRARA N
Corporate Source: GENENTECH INC,DEPT CARDIOVASC RES/S SAN FRANCISCO//CA/94080; GENENTECH INC,DEPT CARDIOVASC RES/S SAN FRANCISCO//CA/94080; GENENTECH INC,DEPT MOLEC BIOL/S SAN FRANCISCO//CA/94080; GENENTECH INC,DEPT MED & ANALYT CHEM/S SAN FRANCISCO//CA/94080
Journal: JOURNAL OF BIOLOGICAL CHEMISTRY, 1992, V267, N36 (DEC 25), P 26031-26037
ISSN: 0021-9258
Language: ENGLISH Document Type: ARTICLE
Abstract: The vascular endothelial growth factor (*VEGF*) family encompasses four polypeptides that result from alternative splicing of mRNA. We have previously demonstrated differences in the secretion pattern of these polypeptides. Stable cell lines expressing VEGFs were established in human embryonic kidney CEN4 cells. VEGF121, the shortest form, was secreted and freely soluble in tissue culture medium. VEG189 was secreted, but was almost entirely bound to the cell surface or extracellular matrix. VEGF165 displayed an intermediary behavior. Suramin induced the release of VEGF189, permitting its characterization as a more basic protein with higher affinity for heparin than VEGF165 or VEGF121, but with similar endothelial cell mitogenic activity. Heparin, heparan sulfate, and heparinase all induced the release of VEGF165 and VEGF165 suggesting heparin-containing proteoglycans as candidate *VEGF*-binding sites. Finally, VEGF165 and

VEGF189 were released from their bound states by treatment with plasmin. The released 34-kDa *dimeric* species are active as endothelial cell mitogens and as vascular permeability agents. We conclude that the bioavailability of *VEGF* may be regulated at the genetic level by alternative splicing that determines whether *VEGF* will be soluble or incorporated into a biological reservoir and also through proteolysis following plasminogen activation.
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